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JOURNAL OF THE AMERICAN PHARMACEUTICAL ASSOCIATION

JUSTIN L. POWERS, EDITOR, WASHINGTON, D. C.

VOLUME XXXVI | *JANUARY, 1947* | NUMBER 1 CONSECUTIVE NO. 2

A Photoelectric Colorimetric Assay for Digitoxin by Comparison with a Standard Powder*,†

By ARTHUR E. JAMES, FRITZ O. LAQUER, and JOSEPH D. McINTYRE

The so-called Keller–Kiliani reaction consists of a series of characteristic colors which develop when 1 to 2 mg. of digitoxin dissolved in glacial acetic acid containing a trace of ferric chloride is underlaid with concentrated sulfuric acid. After a few seconds the acetic acid layer turns to a green color and later to a blue. Optimal conditions for this color reaction were determined, and a photoelectric-colorimetric assay for digitoxin by comparison with a standard powder was developed.

Digitoxin is acknowledged as the most potent glycoside of *Digitalis purpurea*. The announcement that this glycoside is to be official in the forthcoming edition of the United States Pharmacopœia has stimulated new efforts to establish a simple quantitative chemical assay for its standardization thereby avoiding absolute dependence on the expensive and difficult cat bioassay.

It is not within the scope of this paper to deal with the controversial problem of the uniformity, purity, and chemical properties of digitoxin; nor do we attempt to present a complete survey of the voluminous and contradictory literature which has been published about digitoxin. Our efforts have been directed to the development of a simple chemical method whereby the chemist or the pharmacist in a pharmaceutical control laboratory can determine, quantitatively, the digitoxin content of commercial preparations, of tablets, of ampuls, etc. It has long been the desire of the scientific staffs of the control laboratories of pharmaceutical manufacturers to find a procedure for the quantitative chemical determination of digitoxin which can be performed with small amounts of material in a short time.

Several characteristic color tests for the cardiac glycosides of the digitalis group, some of which include quantitative methods. based on these color reactions, have been published. The reviews of Weese (1) and Lillig (2) contain rather complete reports of these color tests. Among these, the Baljet reaction (3) shows that cardiac-active glyco-

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Presented to the Scientific Section, A. PH. A., Pittsburgh meeting, 1946.

† The powder used as Standard and adopted for comparison in this investigation was prepared by the Ladox Laboratories, Inc. It is anticipated that the U. S. P. Revision Committee will issue a Standard Digitoxin in the near future.

sides develop an orange color in contact with alkali salts of picric acid. Using this fact Knudson and Dresbach (4) developed a quantitative assay for digitalis preparations. More recently, Bell and Krantz (5) have modified this method, using a photoelectric colorimeter, to determine the potency of powdered digitalis leaves in comparison with the cat bioassay and with electrocardiograms obtained from patients afflicted with heart diseases. These authors have also developed this method for the assay of digitoxin in tablets (6).

Another color test for the cardiac glycosides of the digitalis group is the so-called Keller–Kiliani reaction which, for many years, has been described for the identification of these glycosides in New and Nonofficial Remedies (7). This reaction consists of a sequence of characteristic colors which develop within a short period of time when 1 to 2 mg. of digitoxin dissolved in glacial acetic acid containing a trace of ferric chloride is underlaid with concentrated sulfuric acid. Although this reaction has value as a qualitative test, the intermingling of 3 or 4 shades of color within a short time interval is not readily adaptable to quantitative colorimetric procedures. However, in 1906 Cloetta and Fischer (8) sought to use this reaction for quantitative purposes. This approach was elaborated in a thesis by Schneider (9). He attempted to use the uniform green color, which developed when very small amounts of purified glycosides of *Digitalis orientalis* (lanata) were treated with glacial acetic acid, ferric chloride, and sulfuric acid, as an approximate estimation of their concentration.

EXPERIMENTAL

Experimental Basis of Development of a Quantitative Color Reaction—The Keller–Kiliani reaction, as performed, presents a sequence of colors developed by the action of concentrated sulfuric acid on a solution of 1 to 2 mg. of digitoxin in about 2 ml. of glacial acetic acid containing traces of ferric chloride. However, if smaller amounts of digitoxin, about 0.1 mg., are dissolved in 2 ml. of glacial acetic acid which contains about 1% of ferric chloride U. S. P. test solution, and a few drops of concentrated sulfuric acid are added, a green to blue color develops which reaches a maximum intensity in fifteen to forty-five minutes, and thereafter gradually fades. Based on a study of these reactions, we have developed a photoelectric colorimetric quantitative assay for digitoxin.[1]

Optimal conditions for the concentrations of digitoxin, of ferric chloride, and of sulfuric acid, as well as the time factor, and the selection of solvent were studied. For extracting digitoxin from tablets three common solvents—chloroform, ethyl alcohol, and methyl alcohol—were considered. Chloroform was selected because it appeared to be preferable in certain instances. The red coloring matter used in certain commercial tablets was found to be insoluble in chloroform, and thus the color did not interfere with the colorimetric determinations, whereas it did dissolve in the alcohols and hence spurious colorimetric readings were obtained. If there are no color interferences either of the alcohols may be used for the extraction. In the quantitative determination of digitoxin in tablets it was necessary to determine the most desirable procedure in order to insure complete extraction of the glycoside from the excipients present in the tablets. Hereafter is reported only that part of the procedure which was found, in general, to give satisfactory results. In all cases a standard reference graph was made from a standard digitoxin powder. The unknown powders, tablets, and ampuls were run through the same process. The digitoxin content of these preparations was determined by reference to the standard graph.

Preparation of a Standard Graph for Comparison.—Standard digitoxin powder, in a very finely divided state, was dried to constant weight in an electric oven at 105°. Exactly 20 mg. of this powder was dissolved in 90% ethyl alcohol and made up to a total volume of 100 ml. Using a blood pipette calibrated in hundredths of a ml., separate samples containing from 0.02 to 0.27 mg. of digitoxin were measured into matched 10-ml. absorption tubes having a light path of 12 ± 1 mm. These tubes were placed in an electric oven at 105° until the alcohol completely evaporated. To each tube containing the various amounts of digitoxin, were successively added exactly 3 ml. of glacial acetic acid, 0.10 ml. of 5% $FeCl_3.6H_2O$ (containing 10.18 mg. Fe per ml.[2]) and 0.25 ml. of concentrated sulfuric acid. These particular volumes were chosen as the result of a preliminary study of optimal concentrations to yield a maximum color with a minimum concentration of digitoxin. A blank containing exactly the same volumes of acetic acid, ferric chloride, and sulfuric acid was prepared. The contents of each tube were thoroughly mixed by inverting. Using a Klett-Summerson photoelectric colorimeter[3] and a light filter having a maximum transmission between 500 and 570 millimicrons, the

[1] This assay is the basis of Method II in the "Digitoxin Chemical Assays" adopted by the Thirteenth revision of The United States Pharmacopœia.

[2] The amount of water in samples of $FeCl_3 \cdot 6H_2O$ is somewhat variable. However, it is unnecessary to know the exact strength of the ferric chloride solution provided the same solution is used in assaying unknowns as was used in the preparation of the standard graph.

[3] Any suitable photoelectric colorimeter may be used.

transmission of each tube was measured at fifteen-minute intervals until a maximum reading was obtained. The maximum reading developed within fifteen to forty-five minutes, depending upon the concentration of digitoxin present. The readings were made from the logarithmic scale of the instrument. Due to the great sensitivity of the method it is advisable to run these calibration determinations in duplicate or triplicate. A typical set of data obtained is as follows:

Mg. of Digitoxin	Maximum Colorimeter Reading
Blank	14
0.02	37
0.03	50
0.05	77
0.06	89
0.08	115
0.09	130
0.10	142
0.12	170
0.15	209
0.18	248
0.21	287
0.24	327
0.26	350
0.27	362

The above data are transcribed to graphical form in Fig. 1. The concentration of digitoxin is plotted as abscissa and the corresponding colorimeter readings as ordinate.

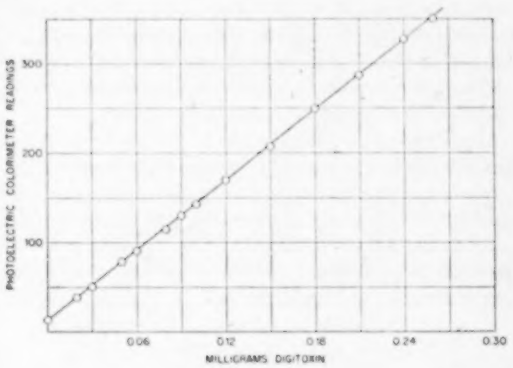

Figure 1.

Assay of Individual Tablets Containing Digitoxin.—Individual tablets, labeled to contain 0.10 mg. or 0.20 mg. digitoxin, were put in 25-ml. test tubes and finely powdered by using a glass rod. The contents of each tube were extracted with 10 ml. of U. S. P. grade chloroform. The supernatant chloroformic solution of each tube was filtered through sintered glass and the residue re-extracted four times with 5-ml. portions of chloroform. The total residues from each six or seven tablets were combined and re-extracted with chloroform to insure the complete extraction of digitoxin. The combined chloroform extract from each tablet, in a matched colorimeter absorption tube, was evaporated to dryness by heating in an electric oven. Care should be used to avoid spattering during evaporation. To each of the tubes containing the chloroform extract obtained from one tablet were added 3 ml. glacial acetic acid, 0.10 ml. of ferric chloride, and 0.25 ml. of concentrated sulfuric acid. Each tube was successively placed in the photoelectric colorimeter and measured at fifteen-minute intervals until a maximum reading was reached. The digitoxin content of each tablet was interpolated from the standard graph. Typical results of different tablets are as follows:

Manufacturer A—Tablets labeled to contain 0.1 mg. Digitoxin:

Maximum Colorimeter Reading	Mg. Digitoxin	
131	0.093	
132	0.093	
144	0.102	
135	0.096	
144	0.102	Av. 0.096
135	0.096	
133	0.095	
Combined residues 15	0.000	

Another manufacturer's tablets, labeled to contain 0.2 mg. digitoxin, yielded values averaging 0.207 mg. per tablet in an assay of six tablets.

A third manufacturer's tablets, labeled to contain 0.2 mg. digitoxin per tablet, averaged 0.210 mg. in an assay of six tablets.

Assay of Lots of 20 Tablets Containing Digitoxin.—Lots of 20 tablets, labeled to contain 0.20 mg. of digitoxin, were finely powdered and let steep overnight in 50 ml. of U. S. P. grade chloroform. The supernatant chloroformic solution was filtered through sintered glass. The residue was re-extracted six times with 5-ml. portions of chloroform. The combined filtrates were made up to 100 ml. with chloroform. Five-milliliter aliquot portions were taken for analysis, evaporated to dryness, and treated as in the case of the assay under single tablets. A final extraction of the residues was performed by letting them steep in 50-ml. chloroform overnight, filtering, and taking 5-ml. aliquot portions for analysis.

The results of three separate lots of 20 tablets are as follows:

Lot	Maximum Colorimeter Reading		Mg. Digitoxin per Tablet	% Extraction,[4] Total
1	264	1st extraction	0.192	
	26	2nd extraction	0.009	100.5
2	254	1st extraction	0.185	
	29	2nd extraction	0.012	98.5
3	260	1st extraction	0.189	
	23	2nd extraction	0.008	98.5

Assay of Ampuls Containing Digitoxin.—The contents of ampuls labeled to contain 0.2 mg. "Digitoxin Injection" in a sterile solution of 40% to 50% alcohol were transferred to matched 10-ml. absorption tubes. The content of each tube was evaporated to dryness in an electric oven at 105°. To each tube were successively added 3 ml. glacial acetic

[4] Based on the labeled content.

acid, 0.10 ml. of 5% ferric chloride, and 0.25 ml. of concentrated sulfuric acid. The maximum colorimeter reading of each tube was measured as described under the assay of digitoxin tablets.

When, however, in addition to dilute alcohol, glycerin is present in the ampul it is necessary to determine the amount of glycerin. This necessitates the preparation of another standard graph for comparison in which corresponding amounts of glycerin are added to the various known weights of digitoxin. Ampuls containing glycerin which were labeled to contain 0.20 mg. digitoxin in 40% alcohol gave the following results: 0.220, 0.194, 0.208, 0.190, and 0.210 mg. per ampul. Average: 0.204 mg. per ampul.

DISCUSSION OF RESULTS

The calibration graph as shown in Chart I exhibits close adherence to Beer's Law. This indicates that the intensity of the greenish blue color, developed under controlled conditions, is in direct proportion to the concentration of digitoxin present. Consequently, by reference to the calibration graph, the amount of digitoxin in commercial preparations may be determined, after proper extraction, by a procedure identical to that which was used in establishing the standard graph. In using the photoelectric colorimeter some laboratories will prefer to set the instrument at zero, 100% light transmission, for the blank containing the acetic acid, ferric chloride, and sulfuric acid. This will yield a graph beginning at the intersection of the abscissa and ordinate.

For practical purposes, this chemical method has been found adequate for the assay of digitoxin. However, attention should be called to the fact that certain other glycosides of the digitalis group may give identical colorimetric values. The relationship of these values to their potency as determined by biological assay is a matter for future study. Therefore, at the present time the application of our method is limited to the determination of digitoxin in commercial preparations which have been prepared from material which meets the specifications for digitoxin as required by the forthcoming thirteenth edition of the United States Pharmacopœia.

A number of samples of digitoxin used in these experiments were bioassayed on cats in accordance with the method described in U. S. P. XII. The results of these bioassays are in close agreement with the values obtained by our colorimetric method and will be the subject of a later publication.

The quantitative determination of digitoxin in tablets or ampuls, by means of the photoelectric colorimetric method previously described, is satisfactory in that it enables one to determine the digitoxin content of a single tablet or ampul containing as low as 0.1 mg. with a deviation of ± 10%. This method is also applicable to the officially recognized practice of using not less than twenty tablets and taking an aliquot portion of the extractive for the actual determination. A deviation of less than 5% was found in this work.

For comparative purposes, the reference used should be a sample of the digitoxin from which the tablets or ampuls were manufactured. When digitoxin becomes official in the U. S. P. XIII, the reference standard used in each laboratory should be checked against the U. S. P. Reference Standard.

In assaying digitoxin tablets, the accuracy of the final results depends upon the complete extraction of the digitoxin. An extraction of from 90% to 100% has been accomplished repeatedly. In a few cases when tablets manufactured by certain pharmaceutical houses were assayed, some variation has occurred due to the nature of filler ingredients in the tablet. Since the amount of digitoxin per tablet is approximately 0.1% to 0.2% of the total, it is readily apparent that small amounts of digitoxin can be so tenaciously adsorbed by the filler material that further treatment with the solvent may be necessary to insure complete extraction.

It appears that the time factor involved in the extraction of digitoxin from tablets is of importance. Tablets which had been pulverized and let stand in chloroform for several hours gave better results than those which had been in contact with the solvent for only an hour or so. The use of a mechanical shaker to extract the digitoxin content of the tablets may be preferred by some laboratories. However, we found that this did not give as satisfactory results as the method we describe. The applicability of this method in determining the potency of leaf, tincture, and partially purified preparations derived from digitalis, which are usually sold under different trade names, is now under study.

Photoelectric colorimetric technique requires considerable experience to obtain concordant results. Hence, it is suggested that those who are unfamiliar with the use of these instruments give careful attention to the details involved. The sensitivity of this method is so delicate that it is desirable to make measurements in duplicate or in triplicate.

CONCLUSIONS

A colorimetric photoelectric method has been developed whereby the U. S. P. XIII digitoxin, tablets, or ampuls may be assayed by comparison with a standard digitoxin reference powder.

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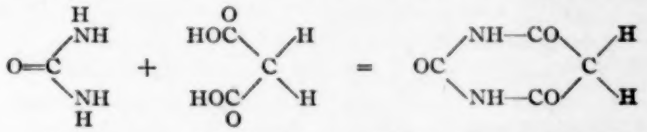
Barbiturates: A Blessing and a Menace*

By SAMUEL W. GOLDSTEIN†‡

The development and therapeutic status of barbituric acid derivatives are reviewed and those in general use as therapeutic agents are tabulated. Data are presented on the incidence of poisonings in the United States by barbiturates. The nature of problems relating to the use and misuse of barbiturates is discussed and the need for corrective uniform state legislation is stressed.

DURING the past forty-two years there have been five outstanding discoveries which have greatly fortified the armamentarium of the physician: (*a*) barbiturates (1903); (*b*) salvarsan (1907); (*c*) insulin (1922); (*d*) sulfonamides (1935), first prepared in 1909; (*e*) antibiotics (1940), although penicillin was first discovered in 1929. No group of drugs caused such chemical activity as the barbiturates until the advent of the sulfonamides.

Here was a combination of urea, the first organic compound to have been prepared synthetically, and malonic acid, a classic example of organic synthesis, to form malonylurea or barbituric acid.



Urea Malonic acid Malonylurea (barbituric acid)

The two hydrogen atoms in the methylene group of barbituric acid are very reactive and can be replaced indirectly by one or two aliphatic or aromatic groups to form compounds having hypnotic properties.

The first of these compounds to be introduced into medicine was the diethyl substituted product, barbital, prepared in 1903 by Emil Fischer and von Mering (1) and patented under the name veronal. This was followed by phenobarbital or luminal which differs from barbital in that one of the ethyl groups is replaced by a phenyl group. The barbituric acid derivatives are only sparingly soluble in water, but the sodium salts, in which sodium replaces a hydrogen atom attached to nitrogen, are freely soluble in water and are referred to as the "sodium barbiturate," the "soluble barbiturate," or the "barbiturate sodium." Starting with thiourea in place of urea a new series of barbiturates has been prepared, the best of which is sodium ethyl (1-methylbutyl) thiobarbiturate or pentothal sodium.

APPROVED BARBITURATES

Over 1500 different barbituric acid derivatives have been prepared, but less than twenty have survived clinical use. Table I lists the clinically useful barbiturates that are recognized in the United States Pharmacopœia XII and New and Nonofficial Remedies 1946, and gives the trade names, synonyms, and chemical names, the approximate relative duration of action, and the average adult hypnotic dose. Barbital was the first of the malonylurea series of drugs to become official in the U. S. P. and is still considered to be one of the best hypnotics. For years the drug manufacturers introduced barbiturates under confusing trade names, each claiming superior virtues for his product with respect to its potency, efficiency or shortness of action, margin of safety, and form and size or color of tablet or capsule. Gardner (2) considers only five barbiturates as essential: Barbital and phenobarbital and their sodium derivatives; pentobarbital sodium; evipal sodium, for intravenous anesthesia; and pentothal sodium, for prolonged intravenous anesthesia. Evipal and pentothal are recognized by the American Medical Association in New and Nonofficial Remedies, while the first three are recognized

* Received Nov. 27, 1946, from the State of Maryland Department of Health, Baltimore.

† Pharmaceutical Chemist, State of Maryland Department of Health.

‡ The author is indebted to the many individuals who cooperated in compiling the data.

in the U. S. P. XII. Pentothal sodium, under the name thiopental sodium, will be included in the U. S. P. XIII which will become official in 1947. Gardner suggests that physicians should limit their barbiturate prescriptions to these five and thereby relieve the pharmacists of the necessity of stocking a large number of named and unnecessary preparations.

are useful in psychic sedation, producing a sound sleep the night before a surgical operation, and for allaying fears just before the operation. The average dose used to produce sleep does not depress the respiration, but large doses can cause death by respiratory failure. Overdoses of barbiturates can indirectly diminish the secretion of urine, and since urine is the most important avenue

TABLE I.—BARBITURATES ACCEPTED FOR CLINICAL USE

Trade Names, Synonyms, and Chemical Names	Recognized in	Duration of Action (Approx.)[a]	Average Adult Hypnotic Dose in Gm.
Alurate[b] Allylisopropylbarbituric acid	N. N. R.	M	0.065–0.13
Amytal[b] Isoamylethylbarbituric acid	N. N. R.	M	0.1–0.3
Barbital[b,c], Barbitone, Veronal Diethylbarbituric acid	U. S. P.	P	0.3
Dial Diallylbarbituric acid	N. N. R.	P	0.1–0.3
Hexobarbital Soluble, Evipal Sodium, Evipal Soluble Sodium N-methylcyclohexenylmethylbarbiturate	N. N. R.	VS	.....
Ipral Calcium,[b] Probarbital Calcium Calcium ethylisopropylbarbiturate	N. N. R.	P	0.12–0.25
Neonal, Soneryl, Butobarbital *n*-Butylethylbarbituric acid	N. N. R.	P	0.05–0.1
Nostal Isopropyl-β-bromallylbarbituric acid	N. N. R.	P	0.1–0.3
Ortal Sodium Sodium *n*-hexylethylbarbiturate	N. N. R.	M	0.2–0.4
Pentobarbital Sodium, Nembutal Sodium ethyl(1-methylbutyl)barbiturate	U. S. P.	M	0.1
Pentothal Sodium, Thiopental Sodium[d] Sodium ethyl(1-methylbutyl)thiobarbiturate	N. N. R.[e]	VS	.....
Pernoston[b] *sec.*Butyl-β-bromallylbarbituric acid	N. N. R.	M	0.2
Phanodorn, Cyclobarbital Cyclohexenylethylbarbituric acid	N. N. R.	M	0.1–0.2
Phenobarbital,[b] Phenobarbitone, Luminal Phenylethylbarbituric acid	U. S. P.	P	0.03–0.1
Sandoptal Isobutylallylbarbituric acid	N. N. R.	M	0.2–0.4
Seconal Sodium Sodium allyl(1-methylbutyl)barbiturate	N. N. R.	S	0.1–0.2
Vinbarbital Sodium, Delvinal Sodium Sodium ethyl(1-methyl-1-butenyl)barbiturate	N. N. R.	M	0.1–0.2

[a] Duration of Action: M = Moderate; P = Prolonged; S = Short; VS = Very Short (intravenous)
[b] Sodium salt also in N. N. R. or U. S. P.
[c] Barbital Sodium also known as Medinal.
[d] Thiopental Sodium in U. S. P. XIII.

The medicinal use of the barbiturates is chiefly for the depression of the central nervous system. Depending upon the dose administered and the patient's reaction, the hypnotic effect can vary from a light sleep to a deep coma. The proper oral dose should induce sleep in twenty to sixty minutes. The barbiturates are used to inhibit convulsions as in strychnine poisoning, tetanus, and epilepsy. Phenobarbital is the drug of choice for epileptics because of its selective action upon the motor cortex. Barbiturates

for the excretion of these drugs, especially the more stable compounds, the patient's recovery is delayed. Thiobarbiturates and barbiturates with complex cyclic radicals, e.g., evipal, are less stable and almost completely destroyed in the liver. McNally (3) states that individuals with impaired hepatic function have remained deeply anesthetized for long periods of time from a hypnotic dose of evipal, which in a normal person would have caused an anesthesia for only fifteen minutes. He also states that the longer-

acting barbiturates should not be given to patients with kidney dysfunction, because failure of the kidneys to excrete the barbiturate causes a cumulative toxicity which is noted in cases where this type of drug is used daily. Even in a normal individual a hypnotic dose of a stable barbiturate is detectable in the urine nine days after ingestion of the drug.

McNally warns against the misuse of barbiturates in the field of obstetrics. Gardner recognizes the value of phenobarbital in small doses as a simple sedative, but believes that the barbiturates as a group should be considered hypnotics, and used as such. He fears that many physicians have forgotten that the bromides and small doses of chloral hydrate and chlorobutanol may more efficiently be used as simple sedatives.

The emotional and nervous strain of the unsettled postwar period is making itself apparent by the increase in appeals to the family physician for relief. The barbiturates can be a blessing to individuals undergoing the mental tortures of sleepless nights due to various causes. The layman who experiences this relief and who has not been clearly warned about the possible dangers of intoxication by these drugs will naturally desire to continue using them. The first-time users should have impressed upon them by their physicians the proper use of the barbiturates. The patient's ability to obtain the drug should be curtailed by prescribing small quantities and writing directions for refilling the prescription when necessary. When no specific directions are given these prescriptions should not be refilled.

A vigorous campaign should be instituted by the American Medical Association to educate the physician to his responsibilities in this field of medication before conditions get so bad that public demand will require drastic legislative steps.

Hambourger (4), in 1940 reported that barbiturate addiction accounted for more than 10 per cent of all addiction cases, excluding chronic alcoholism, reported by thirteen hospitals. Two-thirds of the barbiturate addicts who gave information claimed that they became familiar with the drug through a physician. Nearly a third of the addicts for whom the information was recorded developed craving when the barbiturate was withheld. None showed any serious withdrawal symptoms. It is apparent that addiction to barbiturates presents a problem but its seriousness cannot be compared to that caused by narcotics. Even now, after many years of heroic work by Federal agents, the cases of narcotic addiction are far greater than those of barbiturate dependence. The possibility of barbiturate addiction was considered serious enough by the Food and Drug Administration to lead to the ruling which states that the words "may be habit-forming" must appear on all proprietary preparations containing a barbiturate.

Barbiturates were at first thought to be so free from harmful effects that they were prescribed readily in all conditions where drug-induced relaxation or sleep was thought to be desirable. Indeed it is fortunate that with most individuals there is a wide margin between the therapeutic dose and the toxic dose for the barbiturates usually prescribed for oral administration. Ten to fifteen times the therapeutic dose has generally proved fatal although recoveries from massive doses (barbital 150–500 gr., phenobarbital 120–140 gr.) have been reported (4). Nonfatal poisonings from as little as 5 gr. of barbital and 3 gr. of phenobarbital have also been reported. Wide variations in relation to fatal and nonfatal doses have also been noted in hospital records studied by the author. Chronic poisoning is encountered where elimination is slow and cumulative toxic after-effects are manifested in mental and bodily weakness, tremors, and dizziness.

DEATHS FROM BARBITURATES

The increase in deaths caused by barbiturates is evident in the latest figures for the individual states. The available figures from 1936 to 1945, inclusive, are given in Table II. A majority of the states, especially those with large rural populations, show little change in the yearly numbers of deaths caused by barbiturates from 1936 through 1944. Most of the states with large urban populations show increasing numbers of deaths during this period. The

TABLE II.—DEATHS CAUSED BY BARBITURATES: SUICIDAL AND ACCIDENTAL[a]

	1936	1937	1938	1939	1940	1941	1942	1943	1944	1945
Alabama[b]	3	10	4	4	3	2	2	..	4	...
Arizona	..	2	6	4	4	3	3	1	3	...
Arkansas[b]	2	1	1	1	2	3	2	2	1	...
California[b]	21	31	29	32	56	63	52	93	108	193
Colorado[b]	..	3	2	4	2	7	8	2	4	...
Connecticut[b]	6	9	9	10	9	8	8	6	10	...
Delaware[b]	1	..	..	..	..	..	..	..	...	...
Dist. of Columbia[b]	4	5	8	8	5	2	2	..	5	...
Florida[b]	8	10	13	10	12	14	5	8	10	...
Georgia[b]	5	8	3	3	3	6	..	4	2	9
Idaho	2	4	2	1	3	1	1	..	1	...
Illinois	33	63	56	51	70	70	24	31	33	60
Indiana[b]	11	18	12	16	27	17	12	9	14	...
Iowa	4	9	15	9	8	14	5	5	8	9
Kansas[c]	3	4	3	9	11	3	3	3	1	9
Kentucky	1	4	6	5	3	4	4	2	3	6
Louisiana[b]	4	1	4	9	2	4	5	1	2	...
Maine[b]	4	3	1	4	3	5	4	..	3	...
Maryland[b]	4	1	1	1	5	3	5	7	7	9
Massachusetts	22	20	20	22	24	35	26	28	22	42
Michigan[b]	11	14	16	26	28	31	13	14	11	...
Minnesota[b]	8	14	11	9	9	12	12	13	14	11
Mississippi[b]	..	3	..	..	9	12	14	6	3	4
Missouri[b]	6	13	16	12	32	7	4	10	11	...
Montana[c]	2	..	..	1	2	1	1	1	4	...
Nebraska[b]	..	2	1	4	6	3	3	2	1	...
Nevada[b]	3	..	1	2	1	..	1	1	...	...
New Hampshire[b]	1	1	4	2	1	9	3	1	...	...
New Jersey[b]	7	4	7	7	17	13	9	12	11	...
New Mexico	1	3	1	2	3	4	..	3	2	...
New York[b]	57	49	62	58	60	65	73	87	98	...
North Carolina[b]	4	2	1	1	5	2	4	4	4	...
North Dakota[c]	1	..	..	..	3	1	2	..	...	...
Ohio	28	21	45	32	55	71	42	38	38	72
Oklahoma[b]	5	6	6	3	1	2	3	4	5	...
Oregon[b]	5	6	5	4	2	6	5	1	4	10
Pennsylvania[b]	12	15	10	15	18	20	18	21	18	...
Rhode Island[b]	1	1	2	1	1	1	1	1	2	...
South Carolina[b]	3	3	3	3	1	2	..	..	3	...
South Dakota	..	..	1	..	..	..	1	1	...	...
Tennessee[b]	4	5	2	4	2	2	3	1	5	...
Texas	7	6	5	8	12	16	7	13	11	...
Utah[c]	2	6	5	3	1	1	1	..	3	...
Vermont[b]	..	1	2	..	3	1	3	..	1	...
Virginia[b]	1	..	..	2	2	3	1	4	7	4
Washington[b]	16	17	10	5	8	9	7	12	11	15
West Virginia[b]	5	3	5	1	5	7	9	4	7	...
Wisconsin	7	12	19	14	15	17	13	9	6	...
Wyoming	..	1	..	..	..	2	..	..	1	...

[a] From U. S. Public Health Service, National Office of Vital Statistics, and State Statistics.
[b] States had enacted laws, as of October 1, 1945, regulating the sale of barbiturates.
[c] States were exercising control of sale of barbiturates by regulation as of October 1, 1945.

available figures for 1945 show tremendous increases in the number of deaths in eight of the fourteen states represented, while only one state shows a definite decrease. The emotion-stirring years of 1939–1941 show the expected high figures, but the deaths in 1945 indicate new all-time highs in many states. The increasing number of deaths caused by barbiturates appears to be about the same for states with and without legal controls for the distribution of barbiturates. It is evident that the control measures in effect today are not succeeding in halting the upward trend in deaths caused by barbiturates.

NONFATAL BARBITURATE POISONINGS

The only reliable source of data on nonfatal poisonings is the hospital record or case history. Data obtained from hospitals by Hambourger for the period 1928 to 1937, inclusive, and by the author for the period 1940 to 1945, inclusive, are recorded in Table III. In the present study more emphasis was placed on obtaining data from enough hospitals in one city (Baltimore) to

make certain that patients from all social and economic levels would be included. The data indicate that the barbiturate danger is prevalent among all classes of the population.

Where data are available from both the 1928–1937 and 1940–1945 periods it will be noted that the percentage ratio of barbiturate poisonings to total admissions has almost doubled, while the percentage ratio of barbiturate poisonings to all drug poisoning cases has increased tremendously. The data from Michael Reese Hospital and Walter Reed General Hospital do not follow the trend with respect to the percentage ratio of barbiturate poisonings to total admissions, but the percentage ratio of barbiturate poisonings to all drug poisonings remained the same in the first hospital while the figure of 42.9 per cent for the second hospital shows that almost half its poisoning cases were caused by barbiturates. The data from Johns Hopkins Hospital show that the percentage ratio of barbiturate poisonings to total poisonings for the 1940–1945 period is lower than for the 1936–1937 period. A yearly breakdown shows that this ratio increased each year from a low of 0.5 per cent in 1940 to a high of 21.2 per cent in 1945.

Applying the percentage ratios of barbiturate poisonings to total admissions obtained for the 1940–1945 period (excluding Boston City Hospital) to the same number of admissions for each corresponding hospital as reported in the earlier survey, we obtain the following figures: total admissions 786,766; barbiturate poisonings for earlier period 182; barbiturate poisonings for later period 339. This gives a composite percentage ratio of 0.023 for the 1928–1937 period as compared with a composite percentage ratio of 0.043 for the 1940–1945 period, or an increase of over 86 per cent in the number of barbiturate poisoning cases. In the case of

TABLE III.—ACUTE POISONING BY BARBITURATES

Hospital	1928–1937, Inclusive					1940–1945, Inclusive				
	Hospital Admissions, All Causes (A)	Poisoning Cases Except CO and and Alc. (P)	Barbiturate Poisoning (B)	Percentage of All Poisoning Cases, $\frac{B}{P}$ 100	Percentage of All Admissions, $\frac{B}{A}$ 100	Hospital Admissions, All Causes (A)	Poisoning Cases Except CO and Alc. (P)	Barbiturate Poisoning (B)	Percentage of All Poisoning Cases, $\frac{B}{P}$ 100	Percentage of All Admissions, $\frac{B}{A}$ 100
Boston City	331,481	2214	391	17.7	0.118	163,469[a]	950	271	28.5	0.166
Peter Bent Brigham, Boston	43,885	214	26	12.1	0.059	.....	...	...	..	...
Cleveland City	129,983	..	60	..	0.046	74,709	311	92	29.6	0.123
University Clinics, Chicago	63,076[b]	117	18	15.4	0.028	40,339	55	15	27.3	0.037
University Hospitals, Cleveland	91,703	245	23	9.4	0.025	.....	...	...	..	...
St. Mary's, St. Louis	75,024	99	18	18.2	0.024	52,516	110	18	16.4	0.034
Michael Reese, Chicago	142,067	169	34	20.1	0.024	102,026	40	8	20.0	0.008
Presbyterian, New York	135,352	444	22	5.0	0.016	139,499	193	45	23.3	0.032
Johns Hopkins, Baltimore	32,703[c]	27	5	18.5	0.015	118,407	824	27	3.3	0.023
Baylor University, Dallas, Texas	109,138	293	15	5.1	0.014	111,533	103	50	48.5	0.045
Baltimore City	25,356[d]	114	3	2.6	0.012	37,191	235	19	8.1	0.051
Walter Reed General, Washington, D. C.	74,067	..	7	..	0.009	35,755[e]	7	3	42.9	0.008
Union Memorial, Baltimore	.....	..	...	..	...	32,665	90	29	32.2	0.089
University Hospital, Baltimore	.....	..	...	..	...	69,287	123	22	17.9	0.032
Sinai, Baltimore	.....	..	...	..	...	39,331	110	12	10.9	0.031
Mercy Hospital	.....	..	...	..	...	43,548	77	8	10.3	0.018

[a] 1940–1943, inclusive.
[b] 1927–1938, inclusive.
[c] 1936–1937, only.
[d] 1935–1937, inclusive.
[e] 1943–1944 only.

Boston City Hospital for which we have data through 1943 only, the percentage ratio of 0.118 for the 1928–1937 period compared with that of 0.166 for the 1940–1943 period shows a 41 per cent increase in the frequency of occurrence of barbiturate poisoning cases.

Applying the percentage ratios of barbiturate poisonings to all drug poisoning cases (except alcohol and carbon monoxide) obtained for the 1940–1945 period (excluding Boston City Hospital) to the same number of total drug poisoning cases for each corresponding hospital as reported in the earlier survey, we obtain the following figures: total drug poisoning cases 1263; barbiturate poisonings for earlier period 115; barbiturate poisonings for later period 337. This gives a composite percentage ratio of 9.1 for the 1928–1937 period as compared with 26.7 for the 1940–1945 period, or an increase of over 193 per cent in the frequency of barbiturate poisonings compared to all drug poisonings. Boston City Hospital shows a percentage ratio of 17.7 for the 1928–1937 period compared with that of 28.5 for the 1940–1943 period, or a 61 per cent increase in the frequency of occurrence of barbiturate poisoning cases.

The hospital data for the 1928–1937 period show that in ten hospitals with total admissions of 1,049,785, barbiturates were responsible for one-seventh of the acute poisonings due to all drugs except alcohol and carbon monoxide. The present survey shows that in fourteen hospitals with total admissions of 1,060,275, barbiturates were responsible for one-fifth of the acute poisonings due to all drugs except alcohol and carbon monoxide.

The conditions existing in the period up to 1937 were responsible for the introduction of a resolution at the June, 1937 meeting of the American Medical Association on the "Evils from Promiscuous Use of Barbituric Acid and Derivative Drugs." The resolution included the following statement: "The evils of these drugs include habit formations, toxic cumulative action, their substitution for alcoholic beverages for drunken episodes, their use for successful as well as unsuccessful suicidal attempts, their improper use being a recognized causative factor in many motor accidents and their improper use being a recognized etiologic factor in some criminal assaults . . . ." If the conditions responsible for the position taken by the A. M. A. in 1937 are reflected in the hospital survey made by Doctor Hambourger at the invitation of the A. M. A. Council on Pharmacy and Chemistry, then certainly a much more alarming condition is indicated by the present study.

Data obtained from the publications of the United States Department of Commerce, Bureau of the Census, indicate that while the trend for the United States is lower in suicides and fatal poisonings by all solid and liquid poisons, suicides and fatal poisonings by barbiturates are increasing. This is shown in Figures 1 and 2.

These data emphasize the increase in the number of barbiturate poisonings. About the middle of 1945 the Chief Medical Examiner of New York City reported an increase in the number of deaths in the city from consumption of sleeping tablets from a normal (?) of 40 per year to 47 in the last three months. Early in 1946 the New York City hospitals were reporting a death caused by barbiturates every thirty-six hours. Is there any wonder that New York City already has enacted more stringent legislation for the control of these drugs? The use of barbiturates in the United States has increased at such a rate that whereas in 1936 two hundred and thirty thousand pounds were produced, in 1945 the production of barbiturates was five hundred and fifty thousand pounds.

Many lay "pharmacologists" have discovered that if barbiturates are taken with alcohol they can obtain effects ranging from a "cheap drunk" to a "heroin reaction." In the New York Harlem section the effect is called "Wild Geronimo." These people refer to the barbiturates as goof pills, yellow jackets, red dogs, or red birds, depending upon the color of the capsules, and the combination with beer or liquor is called "a bolt and a jolt."

The use of barbiturates by criminals is considered to be of secondary concern since these people make up a small fraction of the population. Of greater concern are the thou-

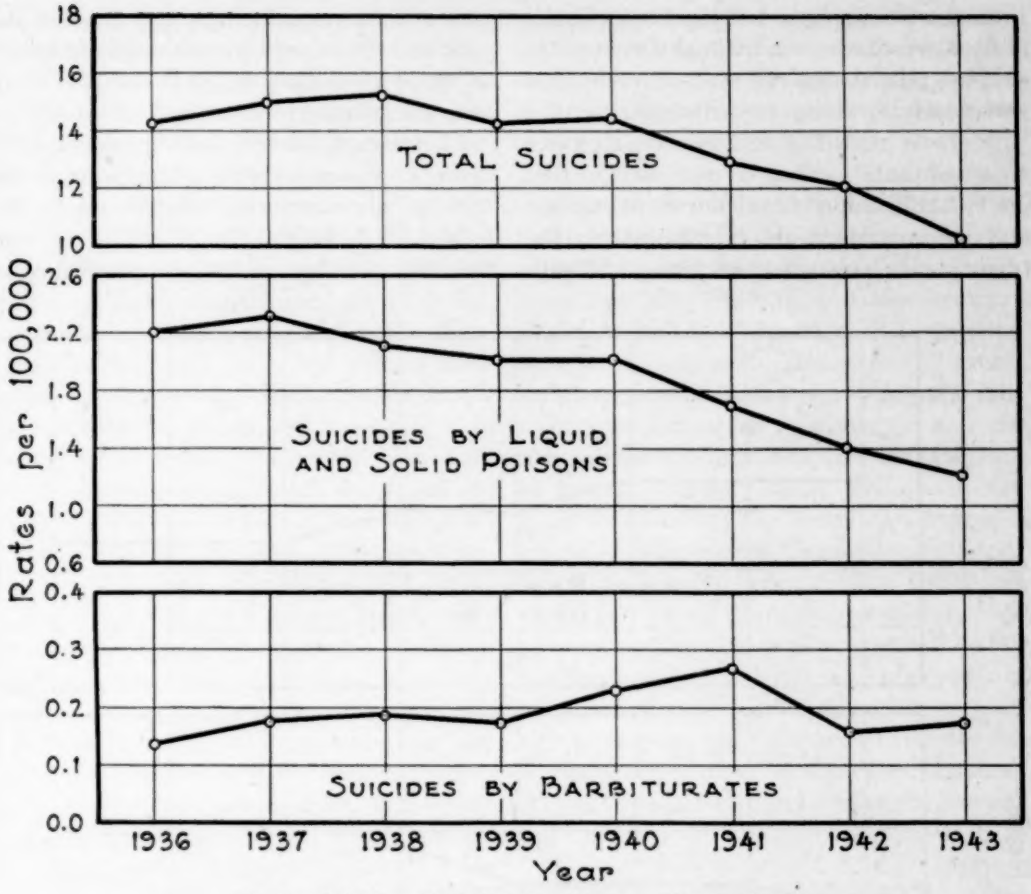


Fig. 1.—Suicides—United States (Bureau of Census Data)

sands of people who regard these compounds as harmless and gradually use them to excess. What is becoming the classic example was discovered not long ago in Waco, Texas. A kindergarten teacher noticed the unusual behavior of a pair of twins. She found that one of them had a box of pills which the child said her father had given to her. Investigation showed that the father, a factory worker, had been put on the night shift. Unable to adjust himself to the new hours he became addicted to sleeping pills. He was still disturbed by the noise of the twins playing, so he started giving them pills too. The children were taking them regularly. This story will be retold many times before adequate control of barbiturates is finally established.

Many accidental cases of barbiturate intoxication are discovered and treated before death takes its toll. Frequently these individuals tell of having taken the usual dose of the drug which did not bring sleep. Then, in the mentally befuddled state induced by the barbiturate, they took more of the drug. How much more they did not know. Barbiturates have now achieved the questionable honor of being chosen as the chemical instrument of suicide, second only to carbon monoxide.

Where can people get more barbiturates than they should have? From physicians; from pharmacists (with prescriptions, and in some cases without prescriptions); from some nurses; from wholesale drug houses; from jobbers; and from individuals who "know the ropes." A strong campaign to educate all physicians, pharmacists, and nurses to the dangers of these drugs should be initiated immediately by the American Medical Association and the AMERICAN PHARMACEUTICAL ASSOCIATION. The public should be made aware of the dangers accompanying misuse of the barbiturates

Prominent newspaper ballyhoo everytime a famous or notorious individual succumbs to sleeping pills should not dramatize the ease with which the victim passed away.

I believe, that if all the unfortunate cases of barbiturate addiction and intoxication due to barbiturates obtained from physicians and pharmacists could be eliminated, the tragic picture presented today would be only

groups have responded as they have in the past and are conscientiously striving to aid in the formulation of legal safeguards to keep the growing monster under control.

Pharmacists should not depend entirely upon those seemingly untiring giants in the field of pharmaceutical advancement Dr. Robert P. Fischelis, Dr. R. L. Swain, and the other members of the group working on

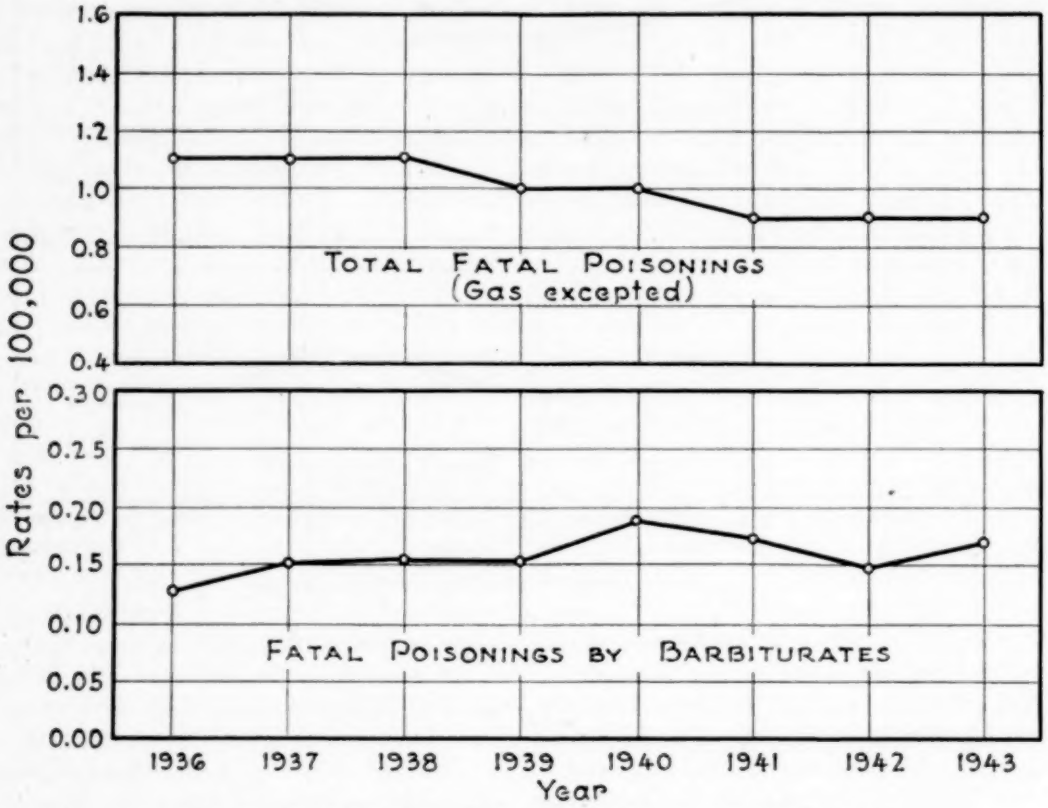


Fig. 2.—Fatal Poisonings—United States (Bureau of CensusDate)

very slightly bettered. Without the knowledge or intent of most manufacturers, wholesalers, dealers, and jobbers, most of the barbiturates that are illegitimately and harmfully used get into the hands of the users through nonprofessional channels.

BARBITURATE CONTROL NEEDED

There can be no doubt that uncontrolled traffic in barbiturates is a growing menace to the public health. Responsible authorities have pointed with alarm and have called for rigid controls of this group of drugs. The leaders of the medical and pharmaceutical

uniform laws affecting pharmacy and medicine. They cannot be expected to achieve results without the active aid and cooperation of the rank and file of the organizations they represent. When A. L. I. Winne of the A. PH. A. Committee on Legislation presented a plan for a uniform state law for the control of barbiturates to the House of Delegates of the AMERICAN PHARMACEUTICAL ASSOCIATION, the most that could be accomplished at that time was a recorded agreement in principle to a uniform state law. More concrete results must be achieved soon or the interested public health groups will find

that they are fighting a losing battle against complete government control. The American Medical Association is now in a position where they are fighting to preserve as much professional freedom as possible in the face of the pressure by certain groups to push through the Congress the modified Wagner–Murray–Dingell bills (S. 1606-H.R. 4730). They still tend to dismiss possible Federal control of barbiturates by saying that the government authorities are not inclined to include barbiturates under the Federal narcotic act. Indeed, Dr. Paul Dunbar, U. S. Commissioner of Foods and Drugs, in discussing Federal control over the distribution of barbiturates and other sleep-producing drugs, stated, "A Federal law would be too complicated and drastic. Uniform state laws would be more satisfactory" (5). Dr. Dunbar's opinion does not mean that a Federal statute regulating barbiturates cannot be passed. Not all Federal and state enforcement officers agree with Dr. Dunbar. A bill (H.R. 6178) was introduced in the Seventy-Ninth Congress by Representative Edith Rogers of Massachusetts that would designate barbiturates as narcotic drugs and place them under all the provisions now effective for the handling of narcotics. This is the first attempt. It will not be the last. It is not surprising that a congressional representative from Massachusetts should be sufficiently interested to do something about the barbiturate situation. Table II reveals that the total deaths caused by barbiturates in Massachusetts in 1945 are 91 per cent higher than the number occurring in 1944. Table III shows that the Boston City Hospital, which has a very large number of admissions, had the highest percentage ratio of barbiturate poisonings to total admissions of any of the listed hospitals. However, it is surprising that the lady's home state, as of October 1, 1945, had not enacted legislation seeking to control barbiturate distribution. Nevertheless, the fact remains that the conditions that have created the demand for such legislation are not improving, as a glance at the data clearly shows.

If physicians and pharmacists would avoid Federal control let them get together on uniform state legislation and do it quickly. Dr. Fischelis' comprehensive review and analysis of the laws pertaining to the control of the distribution of barbiturates, in force in thirty-two states, two possessions, and the District of Columbia as of October 1, 1945, appeared recently (6). This clear picture of the present laws should lend itself to the framing of effective legislation for the control of barbiturate manufacture and distribution. Dr. Fischelis and the members of the A. PH. A. Committee on Legislation have done their part. The points of greatest disagreement as to the wording of a uniform state law seem to center around the renewals of prescriptions and the question of record keeping. Why not utilize the splendid efforts of the AMERICAN PHARMACEUTICAL ASSOCIATION Committee on Legislation up to that point? The individual states can phrase the controversial sections as they see fit. There is no doubt that the phraseology applying to the manufacture and distribution of barbiturates until they reach the physician and pharmacist should be similar in all states. If this cannot be done it might as well be turned over to the Federal authorities.

The problem is here and it is growing. Something must be done to solve it and we should do it as soon as possible.

SUMMARY

1. The development and therapeutic status of the barbituric acid derivatives are reviewed. The accepted clinically valuable barbiturates are tabulated.

2. Deaths caused by barbiturates are shown to be increasing in many states, especially in those states with large urban populations. Where figures for 1945 are available, a sharp increase is indicated for many states. The state laws affecting the distribution of barbiturates that had been passed as of October 1, 1945 appear to have had little effect on the increase in mortality due to barbiturates.

3. The percentage ratio of cases of barbiturate poisonings to total admissions in the same hospitals for the 1928–1937 and 1940–1945 periods show an 86 per cent increase in the frequency of occurrence of barbiturate poisonings in the 1940–1945 period.

4. The percentage ratio of barbiturate poisonings to all drug poisonings (carbon monoxide and alcohol excepted) in the same hospitals for the 1928–1937 and 1940–1945 periods show a 193 per cent increase in the frequency of occurrence of barbiturate poisonings in the 1940–1945 period.

5. Barbiturates accounted for one-seventh of the total drug poisoning cases in ten hospitals with total admissions of 1,049,785 during 1928–1937. Barbiturates accounted for one-fifth of the drug poisoning cases in fourteen hospitals with total admissions of 1,060,275 during 1940–1945.

6. Available data for the United States show that while the yearly cases of suicides and fatal poisonings by all solid and liquid poisons indicate a downward trend, the yearly cases of suicides and fatal poisonings by barbiturates are increasing.

7. The growing nature of the problem is discussed and the urgent need for corrective uniform state legislation is stressed.

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Additional information was obtained from Statisticians at various State Health Departments, from Hospital Officials and Statisticians, and by personal examination of records and case histories in Baltimore City hospitals.

Biologic Assay of Mercury Ointments*,†

By EDWIN P. LAUG, ELIZABETH A. VOS, and FRIEDA M. KUNZE

Using the storage of mercury in the kidney of the rat as a measure of cutaneous penetration, 8 official mercury ointments have been assayed. Mercury oleate and nitrate ointments showed the greatest penetration; calomel and yellow mercuric oxide showed the least. Intermediate in penetration were mercurial, ammoniated and red oxide of mercury ointments. The degree of penetration of mercury was relatively insensitive to large differences in concentration of mercury in the ointments.

REFERENCE to the U. S. P. XII and the N. F. VII reveals that there are 8 different official mercury ointments. The acceptability of these ointments has been judged largely on the basis of either physical properties or the degree of their therapeutic effect. To date, no information has been forthcoming concerning the relative efficiency with which mercury penetrates the skin from these ointments. Recently the authors developed a method (1, 2) for the assay of different types of calomel ointments which was based on the ability of the mercury to penetrate the skin. This method has been applied to the 8 above-mentioned mercury ointments, but with the tacit assumption that skin permeability to mercury is not necessarily the sole criterion of therapeutic efficiency.

* Received Sept. 14, 1946, from the Division of Pharmacology, Food and Drug Administration, Federal Security Agency, Washington 25, D. C.
† A portion of the funds used in this investigation was supplied by a transfer, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Division of Pharmacology of the Food and Drug Administration.

EXPERIMENTAL

Method.—The principle of this method is based on the observation that the concentration of mercury in the kidney is a measure of the amount which has penetrated the skin (1). Briefly, the assay is carried out as follows: Rats are lightly anesthetized, and 0.4 Gm. of mercury ointment is inuncted over an area of 29 $(cm.)^2$ ($1^1/_2 \times 3$ in.) of the clipped dorsal skin. Inunction is made for two minutes with a glass rod. Then, without removal of the excess ointment, the animal is wrapped in a cylindrical celluloid shield cemented to the body at the shoulders and hips. The shield is sufficiently stiff so that the animal is confined after the fashion of a straight jacket, and is thus prevented from

TABLE I.—ASSAY OF OFFICIAL MERCURY OINTMENTS. THE MERCURY CONTENT OF THE KIDNEYS OF RATS IS USED AS A MEASURE OF THE PENETRATION OF MERCURY THROUGH THE SKIN

Mercury Ointment			Mercury Content Kidney, γ/Gm. Wet Tissue
Designation	Source	Mercury Content, %	
Oleate of Mercury	U. S. P. XII, p. 308	23	38
			38
			28
			25
			26
			26
			Av. 30.1
Ointment of Mercuric Nitrate	N. F. VII, p. 470	7.0	22
			27
			18
			17
			11
			23
			Av. 19.7
Strong Mercurial Ointment	U. S. P. XII, p. 536	50	15
			16
			17
			7.5
			6.1
			9.8
			Av. 11.9
Mild Mercurial Ointment	U. S. P. XII, p. 536	10	13
			18
			6.5
			9.7
			8.4
			7.3
			Av. 10.5
Ammoniated Mercury Ointment	U. S. P. XII, p. 536	4.0	18
			14
			7.7
			5.8
			8.8
			11
			Av. 10.9
Ointment of Red Mercuric Oxide	N. F. VII, p. 471	9.3	7.6
			17
			15
			8.6
			10
			11
			Av. 11.5
Yellow Mercuric Oxide Ointment	U. S. P. XII, p. 538	0.93	9.1
			8.4
			9.7
			6.0
			3.3
			5.4
			Av. 7.0
Ointment of Mild Mercurous Chloride	N. F. VII, p. 470	25	8.1
			9.5
			9.0
			4.9
			5.7
			7.7
			Av. 7.5

bending its body or coming in oral contact with it. The shield does not, however, keep the rat from walking about in its cage, nor does it interfere with its intake of food and water. At the end of twenty-four hours the animal is killed, exsanguinated, and the kidneys removed for chemical analysis of mercury (3). Usually 6 animals are used for an assay.

RESULTS

Assay of the 8 ointments is shown in Table I. Using the storage of mercury in the kidney as an index of cutaneous penetration, it can be seen that there are marked differences between ointments. The oleate and the nitrate give a cutaneous penetration of mercury significantly greater than all of the others. The mercurial (strong and mild), ammoniated and red oxide occupy an intermediate position, while the lowest penetration is recorded for yellow oxide and calomel. It is to be noted that although the ointments differ widely in their mercury content, this factor does not appear to determine penetration. Thus, in the case of the strong and mild mercurial ointments, both having the same vehicle but differing by 5-fold in their mercury content, there is no difference in penetration. Even more striking is the fact that calomel and yellow oxide ointments have the same penetration even though they differ by 25-fold in their mercury content. With respect to the effect of the vehicle, it should be pointed out that all except the nitrate and oleate ointments contain very large proportions of petrolatum which has been shown to hinder penetration (2). This may be the effect which reduces the strong mercurial, mild mercurial, ammoniated and red oxide to the same moderately low penetration even though these ointments differ widely in concentration and type of mercury compound. It is not possible from these data to arrive at a definite conclusion concerning the effect of the compound since the number of variables is too large. While the high penetration of the oleate and the nitrate may be accounted for by some intrinsic factor, it should also be remembered that both compounds are suspended in vehicles containing large proportions of free or combined oleic acid. Oleic acid has been shown elsewhere (2) to increase the penetration of calomel markedly.

SUMMARY

Eight official mercury ointments have been assayed biologically. Evaluation of the ointments was based on the degree of cutaneous penetration of mercury in the rat.

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Pharmaceutical Applications of Isopropyl Alcohol. II. Solubilities of Local Anesthetics*,†

By HENRY M. BURLAGE‡

WITH THE inclusion of isopropyl alcohol in The National Formulary, it seems important to examine more fully its pharmaceutical possibilities. This alcohol has many properties similar to ethanol, some of which seem to make it superior to the latter for certain purposes. Also, the fact that it is tax-free is an important consideration in its use as a solvent. Since the solubility data are rather meager or inconclusive, it was advisable to begin by selecting the prominent group of compounds—the local anesthetics.

EXPERIMENTAL

Since most of the solutions of solids employed in pharmacy are weight-in-volume solutions, the solubility data obtained in this study are on this basis (from a practical standpoint). Accordingly, saturated solutions of the compound in the alcohol were prepared at 25° and definite volumes of the solution were measured into tared dishes by means of standard pipettes. The solvent was allowed to evaporate at room temperature and the residue was dried at 105°. In the case of losses due to apparent decomposition, the residue was dried in a desiccator. The results are shown in the following table.

TABLE I.—SOLUBILITIES OF LOCAL ANESTHETICS IN ISOPROPYL ALCOHOL

Name of Compound	Manufacturer	Solubility, Gm./100 Cc. of Solution
Alypin Base (Amydricaine)	Winthrop	Liquid-miscible in all proportions
Alypin Hydrochloride N. N. R. (Amydricaine Hydrochloride)	Winthrop	38.0844
Amylsine Hydrochloride N. N. R. (Amylcaine)	Novocol Chemical Mfg. Co.	0.7768
Aminocaine (Amylcaine)	Seydel	49.5370
Apothesine Hydrochloride N. N. R.	Parke Davis Co.	5.7710
Borocaine (Ethocaine Borate)	Sharp and Dohme	2.3290
Butyl Aminobenzoate U. S. P. (Butesin)	Abbott	47.0840
Butesin Picrate N. N. R.	Abbott	9.2500
Diothane Base	Merrell	38.7700
Diothane Hydrochloride N. N. R.	Merrell	0.5710
Ethacaine	Seydel	36.2570
Ethyl Aminobenzoate U. S. P. (Benzocaine)	Parke Davis Co.	16.8430
(Benzocaine)	Seydel	16.6450
Eucupin Base	Rare Chemicals	34.3030
Eucupin Dihydrochloride	Rare Chemicals	7.8640
Intracaine	Squibb	1.6070
Metycaine Base	Lilly	Liquid-miscible in all proportions
Metycaine Hydrochloride N. N. R.	Lilly	2.9140
Nupercaine Hydrochloride N. N. R. (Dibucaine)	Ciba	Insufficient sample greater than 1 Gm. in 2; 4 cc.
Orthoform N. N. R. (Orthoform-New)	Winthrop	12.0450
Panthesine	Sandoz	1.5160
Phenacaine Base (Holocaine Base)	Winthrop	4.9960
Procaine Base	Abbott	41.8780
Procaine Butyrate	Rorer	69.4800 (unstable)
Procaine Hydrochloride U. S. P.	Abbott	0.6030
Tetracaine Base (Pontocaine Base)	Winthrop	36.6400
Tetracaine Hydrochloride U. S. P. (Pontocaine Hydrochloride	Winthrop	1.5350
Tutocaine Hydrochloride (Butamin)	Winthrop	0.1980

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SUMMARY

The solubilities, in isopropyl alcohol, of 27 local anesthetics on the American market are reported on the basis of weight in volume.

The Effect of Serum and Organ Emulsions on Penicillin Fractions*

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The difference in the degree of inactivation of penicillin fractions by human, rabbit, bovine sera, or rabbit tissue emulsions is not sufficient to establish a differential assay method for estimating the various fraction concentrations in a given sample. In sera, penicillin K is more unstable than the other forms of penicillin, namely G, X, and F.

THERE is considerable interest in the development of methods for differentiating the various fractions of penicillin and particularly for differentiating penicillin G from penicillin K. In view of the reported deficiencies of penicillin K as a therapeutic agent the need for a method for estimating the K content of commercial penicillin is apparent. While the penicillin K content of commercial penicillin may not be especially important where the amount of penicillin given depends on the condition of the patient, public health programs for the treatment of venereal diseases require a standardized dosage schedule which cannot be maintained if the penicillin varies in therapeutic efficacy.

Certain groups directly interested in this field were of the opinion that the failure of penicillin K to maintain levels comparable to those given with penicillin G was due to the presence in the serum of a labile enzyme (1) which was capable of destroying a greater amount of penicillin K than of penicillin G. It was suggested by this group of observers that a study of the action of sera on penicillin might show a point at which inactivation of one fraction would be complete while partial inactivation of the other occurred. The inactivation of penicillin by sera was reported by Bigger (2) in 1944 who stated that penicillin was inactivated by whole blood and serum but that a marked difference in the degree of inactivation existed with different lots of serum. This inactivation according to Bigger was always more pronounced at 37°. The penicillin he employed was an impure preparation and the predominating fraction was not determined although it was probably penicillin F. It was thought that the employment of crystalline fractions of penicillin in a comprehensive study might serve to answer the question whether serum inactivation could be utilized to differentiate penicillin fractions. Consequently the following series of experiments were made to determine the effect of sera on penicillins G, X, F, and K at several temperatures and reaction times. The sera employed represented pools of from two to ten individual serums to reduce variation to a minimum.

EXPERIMENTAL

In conducting the tests the desired quantity of the appropriate crystalline fraction was dissolved in 0.1 ml. of sterile distilled water and the volume brought to 2.0 ml. by adding the serum under test. This represented a 95% concentration of the serum. The 10° temperature was obtained by placing the tubes in a refrigerator while all other temperatures were maintained by placing the tubes in a water bath which had a variation of ±0.5°. After the desired time had elapsed a portion of the mixture was removed and diluted to one unit (estimated) for assay by diluting in sterile phosphate buffer (1%) at *p*H 6.0. The penicillin content was then determined by a modification of the *Staphylococcus aureus* filter-paper disc method of Vincent (3).

DISCUSSION

In conducting these experiments numerous variables were introduced in so far as the time of exposure to serum and the concentration of penicillin was concerned. In the first experiments 10 units of the penicillin fractions per ml. of serum were used and following the suggestion of a second group working on this problem the unitage was raised to 100 and 1000 units per ml. on the basis that higher concentration according to preliminary work done by them resulted in 99.9% destruction of the K fraction with only 80% destruction of the G fraction. The results at these higher concentrations were similar to those obtained at the 10 unit level and though this work did not result in a simple means for differentiating the fractions it did afford useful information concerning the relative over-all stability of the penicillin fractions in sera.

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The data obtained from these studies is presented in condensed form in charts I to VIII (Fig. 1). If the charts are studied as a whole it is apparent that under all of the conditions of these tests penicillin K is by far the most unstable of the four fractions of penicillin and that, in general, the order of instability in sera is K, G, F, and X.

Using rabbit sera at 37° and a concentration of 10 units of penicillin (chart I) the inactivation of penicillin K was complete but under the same conditions the inactivation of penicillin G was 90%; consequently the difference would hardly be sufficient to permit its use as a means of differentiation. When the temperature is raised to 55° with this concentration of penicillin there is no significant change in the order of inactivation which is K>G>F>X.

Chart III shows the degree of inactivation obtained at various time intervals at 37° when the concentration of the penicillins is raised to 100 units per ml. At four, six, and eight hours the relative order of inactivation was K>G>X>F while after twenty-four hours all fractions were inactivated to the same extent. An apparent discrepancy exists when a comparison is made between the per cent inactivated at this temperature when 10 units and 100 units are employed, for one would expect the percentage of penicillin inactivated to decrease as the concentration was increased. The most probable explanation here is, perhaps, the batch to batch variation in the ability of serum to inactivate penicillin. This variation has been reported by Bigger (2) and has been repeatedly observed in our laboratories in serum from human patients held for penicillin assay for more than one day.

Chart IV presents the data obtained when temperatures of 40° and 44° were used with penicillin concentrations of 100 and 1000 units for twenty-four hours.

Chart V represents the effects of human sera on 100 units/ml. of penicillins G and K at 37°. Here again the somewhat more marked instability of penicillin K may be observed. When bovine sera was employed, however, (chart VII) there is a greater destruction of penicillin G at six and eight hours while at twenty-four hours the inactivation is the same for both fractions. The differences between human sera and the bovine sera used is further evidence when the data in chart VI and VIII are considered.

When it became obvious that it was not possible to utilize sera as a means of differential determination of the amounts of penicillin K in commercial penicillin it was decided that tissue emulsions might afford a lead. Abraham, *et al.* (4) had previously found that negligible inactivation resulted when penicillin was incubated with blood, liver, spleen, muscle, brain, lymph nodes, lung and intestine. The type of penicillin with which they were working is not stated but it is highly probable that the predominating fraction was penicillin F.

In these studies tissues were removed from a freshly killed rabbit and 10% emulsions made by homogenizing in a Waring blendor. To 1.9 ml. of this emulsion 0.1 ml. of the appropriate penicillin fraction in water was added to give a final concen-

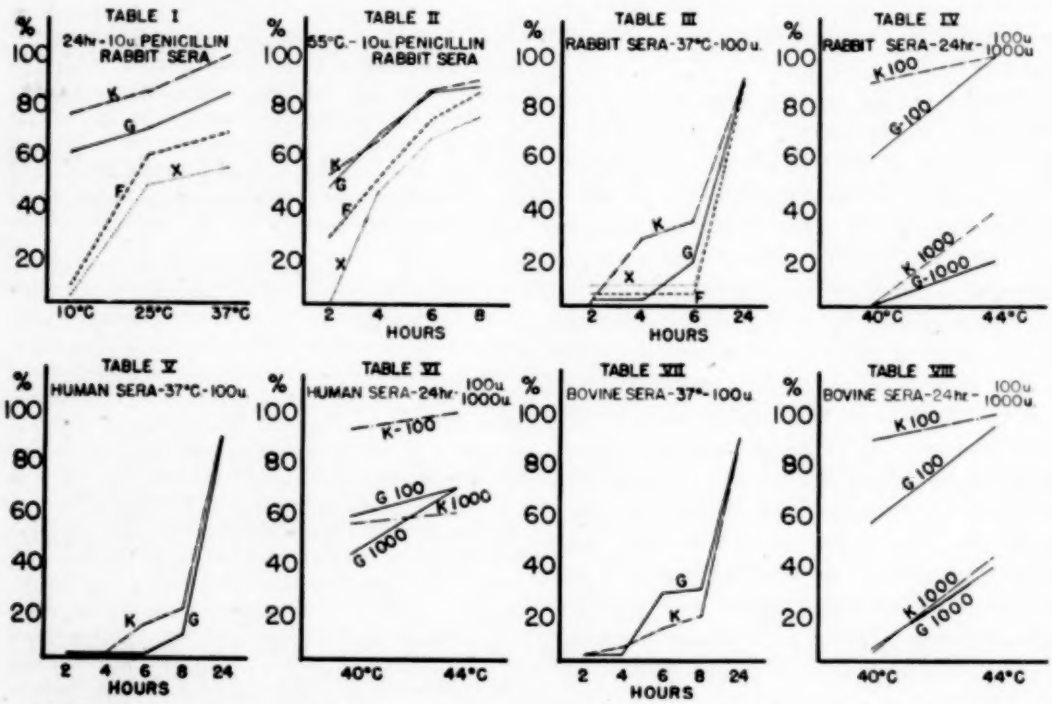

Fig. 1.—The effect of time, temperature, and concentration of penicillin on per cent inactivation of various penicillin fractions. (Referred to in text as "charts" I to VIII.)

TABLE I.—PER CENT INACTIVATION OF CRYSTALLINE PENICILLIN "G" AND "K" USING AVERAGES OF ASSAYS MADE AT 2, 4, 6, AND 24 HOURS

Tissue	Average % Inactivation G	K
Gall bladder	31.0	10.0
Fat	29.5	21.0
Heart	21.0	16.0
Liver	24.0	7.0
Lung	25.5	4.0
Muscle	19.0	12.0
Kidney	19.0	19.0
Spleen	10.0	15.5
Average, All Tissues	22.37	13.06

tration of 10,000 units per ml. This level was not selected because it represents any conceivable concentration which might occur under conditions of actual use in the body but rather because it was decided that unless the inactivating abilities of one emulsion was far superior in selective destruction of one of the fractions it would be of little actual value. The mixtures were incubated in a water bath at 37° and assays made at 2, 4, 6, and 24 hours. In order to simplify the data the results are presented in Table I as averages for the time intervals.

It can be seen from Table I that emulsions of spleen, kidney, muscle, heart and fat inactivated about the same amounts of penicillin K and G. The lungs, liver, and gall bladder inactivated considerably more G than K. A consideration of Table I in its entirety indicates that penicillin G is destroyed by tissue emulsions to a somewhat greater extent than penicillin K.

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The Urinary Excretion of Penicillins G and K in Rabbits*

By C. W. PRICE, W. A. RANDALL, and HENRY WELCH

Results of experiments designed to determine the fate of penicillins G and K from the rate and total amount of urinary excretion of these fractions in rabbits are reported. Comparative data on the rate of excretion, blood levels, and relative effectiveness of penicillins G and K are presented.

IT HAS been reported recently by Coghill, *et al.* (1) that penicillin K is more rapidly excreted by human beings than other penicillin fractions, namely G, X, and a fraction numbered 128. They concluded that penicillin K must be more rapidly destroyed in the body. Other studies have indicated (2) that penicillin K is less effective *in vivo* than penicillin G against several species of organisms and only about one-tenth as effective in the treatment of rabbit syphilis. The latter therapeutic failure has been related to a greater destruction of penicillin K in the intact rabbit with accordingly low total urinary excretion.

Previous experiments (3) on the differential inactivation of penicillin K by serum showed that although somewhat more penicillin K was inactivated by serum than penicillin G, the difference did not seem sufficiently great to explain the reported marked clinical ineffectiveness of penicillin K. In order to throw light on the fate of penicillin G and K in rabbits, experiments were designed to determine the rate and total amount of urinary excretion of these fractions. The penicillins used were obtained through the Antibiotics Study Section of the U. S. Public Health Service.

EXPERIMENTAL

Crystalline penicillins G and K were administered intravenously to rabbits at two dosage levels: 2000 u./Kg. and 20,000 u./Kg. Male rabbits weighing about 3 Kg. were used. The animals were strapped to boards and the heads elevated. Catheters were

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inserted into the bladder and the residual urine removed by lavage with 20 ml. of distilled water followed by the application of pressure to the abdomen above the bladder. After the bladder was emptied the penicillin fraction under test was dissolved in water and injected intravenously in a dose of 2000 u./Kg. or 20,000 u./Kg. using 1.0 ml. of solution per Kg. of body weight. Urines were collected by lavage with 20 ml. of distilled water followed by massage of the abdomen at 15, 30, 60, 90, 120, 150, 180, and 240 minutes after the injection. Dilutions for assay were made in phosphate buffer *p*H 6.0 and the penicillin content determined by the *Staphylococcus aureus* filter disc plate technic.

Blood samples were drawn from each animal by cardiac puncture at the end of thirty or sixty minutes and penicillin levels determined. Thirty-eight rabbits were employed distibuted as follows: 12 received penicillin K at the 20,000 unit level; 10 received penicillin G at the 20,000 unit level; 9 received penicillin K at the 2000 unit level and 7 received penicillin G at the 2000 unit level. The results obtained are shown in Tables I and II and are graphically presented in Fig. 1.

TABLE I.—AVERAGE CUMULATIVE PER CENT EXCRETION OF PENICILLINS G AND K

Time Interval, Min.	Penicillin G, 20,000; Per Cent	Penicillin K, 20,000; Per Cent	Penicillin G, 2000; Per Cent	Penicillin K, 2000; Per Cent
15	18.2	15.3	19.3	19.0
30	24.9	41.6	39.7	36.8
60	45.2	55.4	50.7	44.1
90	52.0	60.3	54.1	46.4
120	57.1	61.5	56.6	47.1
150	57.7	61.7	57.2	47.3
180	59.6	62.1	57.6	47.6
240	59.9	62.4	57.8	47.8

TABLE II.—BLOOD LEVELS OF PENICILLINS G AND K

Penicillin	Level, u./Kg.	Time, Min.	Range	Average
K	20,000	60	0.2–1.3	0.59
G	20,000	60	0.8–2.8	1.52
K	2,000	30	0.0–0.0	0.0
G	2,000	30	0.0–0.32[a]	0.045

[a] One rabbit only gave a level; all others 0.0.

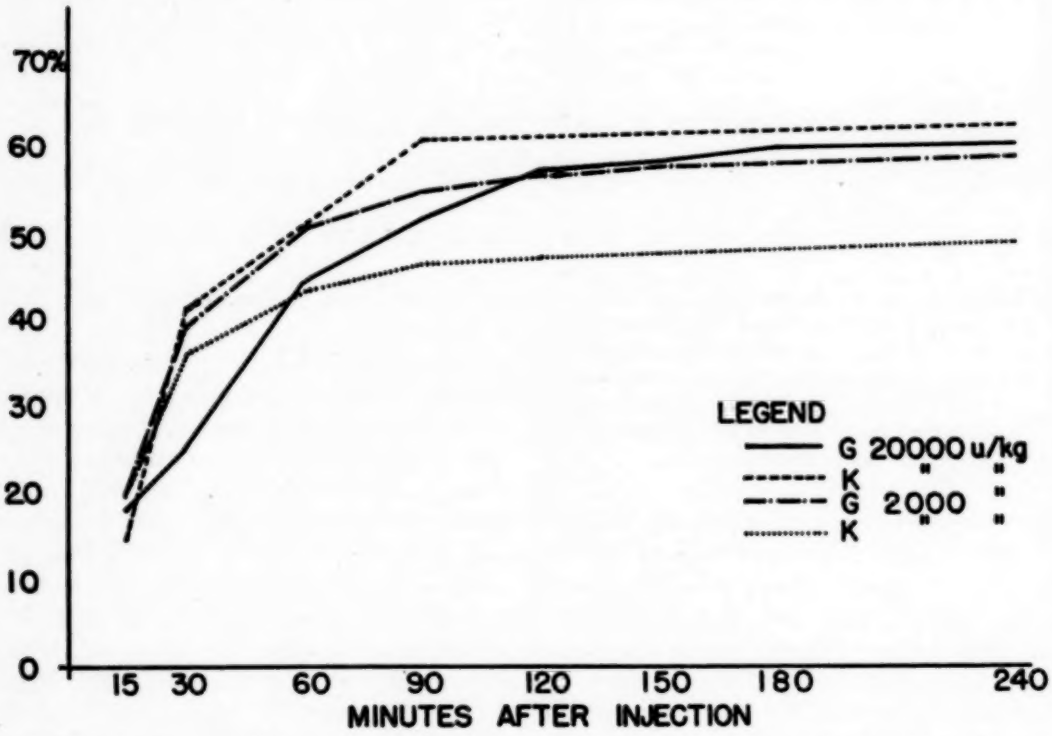

Fig. 1.—Average cumulative per cent urinary excretion following intravenous injection of penicillins G and K in rabbits.

The average cumulative per cent excretion of penicillin G and K at the 20,000 units per Kg. level indicate that the excretion of penicillin G and K occurs at a different rate during the first half hour following injection (Table I). During this period an average of 44.6% of the penicillin K was excreted as compared with 24.9% of penicillin G. The individual values for the twelve rabbits used for penicillin G were from 4% to 56% at thirty minutes while the values for the 10 rabbits used for K varied from 15% to 65% in the same time period. During the next thirty minutes penicillin G is excreted at a faster

rate than penicillin K and from then on the excretion rate is about the same for both fractions.

When 2000 units per Kg. are injected under the same conditions this increased excretion during the first thirty minutes was not observed. After thirty minutes the total amount of penicillin K excreted during any given period is approximately 10% less than the amount of penicillin G. It is of interest that the total amount of penicillin G and K excreted when 20,000 units per Kg. were injected was about the same. Similarly the percentage of penicillin G excreted when 2000 units per Kg. were given was the same as the per cent penicillin G and K excreted at the 20,000 unit per Kg. level while the percent penicillin K excreted at the 2000 unit per Kg. level was only 10% less.

The penicillin blood levels of the animals injected with 20,000 units per Kg. or 2000 units per Kg. are shown in Table II. The average blood level of the rabbits receiving 20,000 units per Kg. of penicillin G was 1.52 units per ml. at the end of sixty minutes which is approximately three times the figure obtained for penicillin K (0.59 unit per ml.) in the same time period. This may be explained by the increased excretion of penicillin K during this period as well as by the greater inactivation of this fraction *in vivo*. Following the injection of 2000 units per Kg. of the two fractions no penicillin levels were demonstrable at the end of thirty minutes in the case of penicillin K, while none or low levels were obtained with penicillin G. It should be pointed out, however, that the *Staph. aureus* filter disc plate method of assay will only detect penicillin concentrations greater than 0.15 unit per ml. The total urinary excretion of penicillin K in man has been reported to be 28% when 25,000 units were given intravenously (1, 2). In the rabbit experiments reported here about 60% of the total dose of both G and K was excreted in four hours, when 20,000 units per Kg. were given. It was thought that this relatively high dose might account for the high total excretion in rabbits and that if large amounts of penicillin K were given to humans the total amount excreted might be greater. Accordingly two healthy adult males were given a single injection of 1,000,000 units of penicillin K intramuscularly. In each case the total amount excreted, in agreement with that reported by Coghill, *et al.* (1), was approximately 30%. A point of interest was the duration of the urine penicillin levels. The collection of urine samples for assay was terminated in one case at the 19th hour and in the second case at the 32nd hour. In the former case there were 2 units of penicillin per ml. of urine at the 19th hour and in the latter case 1.2 units per ml. were found at the 32nd hour. The low total excretion in human beings is in contrast to the results obtained in rabbits and perhaps indicates a more effective mechanism in man for the inactivation of penicillin K. In order to determine whether this greater inactivation of penicillin K was accompanied by a marked decrease in clinical effectiveness, eleven patients with gonorrhea were treated with various dosage levels of penicillin K, and for comparison eight patients were treated with crystalline penicillin G. Table III shows the total number of units which were given in four evenly divided doses every hour for four hours and the therapeutic response obtained.

TABLE III

Patient	Type of Penicillin	Total Amount of Penicillin Injected, Units	Therapeutic Result
1	K	36,000	Cured
2	K	55,000	Cured
3	K	58,000	Failure
4	K	37,000	Failure
5	K	56,000	Cured
6	K	200,000	Cured
7	K	200,000	Cured
8	K	200,000	Cured
9	K	200,000	Cured
10	K	200,000	Cured
11	K	200,000	Failure
12	G	107,000	Cured
13	G	78,000	Cured
14	G	113,000	Cured
15	G	108,000	Cured
16	G	200,000	Cured
17	G	200,000	Cured
18	G	200,000	Cured
19	G	200,000	Cured

It can be seen that in this small series of cases although penicillin K does not appear to be as effective as penicillin G in the treatment of gonorrhea it still has considerable therapeutic value. Of the eleven patients treated with various amounts of penicillin K there were only three failures. If only those patients receiving 200,000 units are considered there was only one failure out of the six treated with this amount. It should be noted that one patient was cured by as little as 36,000 units of penicillin K.

From the evidence now available it is obvious that penicillin K is not as effective clinically as either of the penicillin fractions G or X. On the basis of preliminary data manufacturers have, for the past several months, markedly decreased their over-all penicillin production by cutting down the production of penicillin K through the use of precursors that stimulate the penicillia to produce preferentially the G fraction. During these past few months considerable emphasis has been placed upon the lack of clinical effectiveness of penicillin K, perhaps more than the facts warrant. There is no question but that penicillin K is considerably less effective than penicillin G in the treatment of rabbit syphilis, and it is probable that a similar situation exists in the treatment of this disease in men. However, in other diseases, those caused for example by the gonococcus, the pneumococcus, the streptococcus and possibly others, penicillin K cannot be written off as an ineffectual drug. If, in the treatment of a pneumococcus or streptococcus infection, a daily dose of 300,000 units of penicillin G is utilized, and the data so far indicates that penicillin K is one-third to one-fifth as

effective as penicillin G, it would require from 900,000 to 1,500,000 units of penicillin K to treat such cases. In terms of "units" these latter doses appear to be relatively large. On a weight basis an entirely different picture is presented since in the case of penicillin G 3 gr. per day would be given in the treatment of such diseases and in the case of penicillin K from 9 to 15 gr. per day. In view of the tremendous spread between the therapeutic and toxic doses of penicillin the dose of penicillin K given above on a weight basis is infinitely small. In the case of gonorrhea the comparison is even more striking. In most cases of gonorrhea successful cures are obtained with 100,000 units of penicillin G (1 gr.). If, in gonorrhea, penicillin K is only one-half to one-third as effective, 2 to 3 gr. of this penicillin fraction would be required to obtain results comparable to those obtained with the G fraction. There is no question but that the problem in the social diseases is somewhat different than that of the acute infections, such as pneumonia or streptococcus infections and that it is very desirable to set up a specific relatively rigid dosage schedule for the former diseases. There is no reason why this cannot be done in view of the present availability of crystalline penicillin which consists largely of the G fraction. Actually, however, there is no reason why even penicillin K could not be used effectively in the treatment of syphilis provided the dose were made sufficiently high. If we can assume in syphilis in man that the factor of effectiveness of G to K is 10 to 1, in the case of penicillin G, 2,400,000 units would be given over a period of eight days while in the case of penicillin K 24,000,000 units would be required. The massiveness of "this dose" is also considerably minimized when placed on a weight basis since in the case of penicillin G approximately 3 gr. of this drug would be given daily in the syphilis dosage schedule and only 30 gr. daily of penicillin K. These latter doses cannot be considered as massive when compared with the dosages of arsenicals and bismuth that have been prescribed for this disease, particularly when consideration is given to the wide range between the therapeutic and toxic doses of the various penicillin fractions. On the basis of the data now available it appears that pencillin K cannot be considered an ineffectual therapeutic agent regardless of the diseases against which its antibiotic effect is directed and that extensive clinical studies are necessary to evaluate properly the clinical effectiveness of this penicillin fraction.

CONCLUSIONS

1. At dosage levels of 20,000 u /Kg. in rabbits, penicillin K is excreted at a faster initial rate than penicillin G but thereafter the excretion rate is about the same.

2. When 2000 units of penicillin K per Kg. are injected into rabbits this faster initial excretory rate is not observed and the total excretion of penicillin K is about 10% less than that of penicillin G. In man, however, the total excretion of penicillin K is about one-half that observed in rabbits.

3. Blood levels at sixty minutes are about 3 times as high with penicillin G than with the K fraction when 20,000 u./Kg. is injected into rabbits.

4. When 2000 u./Kg. were injected penicillin K did not give detectable blood levels at the end of thirty minutes while very low average levels were obtained with the same dose of penicillin G.

5. Although penicillin K is not as effective as penicillin G in the treatment of gonorrhea the evidence indicates that it still has considerable therapeutic value in this disease.

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The Effect of Renal Ligation on Serum Penicillin Levels Produced by Penicillins G and K in Rabbits*

By HENRY WELCH, C. W. PRICE and W. A. RANDALL

Evidence is presented that in rabbits complete renal ligation results in prolongation of blood levels following the injection of either penicillin K or penicillin G. A theory is advanced that both excretion and an undetermined inactivation mechanism are involved in the failure of penicillin K to maintain adequate blood levels in rabbits. This inactivation mechanism is believed also to inactivate penicillin G but to a lesser extent than penicillin K.

IN THE past few months much evidence has accumulated indicating that penicillin K is clinically inferior to penicillin G when administered in comparable doses. When tested *in vitro* the activity of penicillin K is more pronounced than penicillin G against numerous organisms and when it is assayed by the official F.D.A. cup-plate method against *Staphylococcus aureus* potencies of from 2200 to 2300 units per milligram of dry material are obtained as against the standard crystalline penicillin G which has a potency of 1667 units per milligram. Utilizing turbidimetric assays, values as high as 2600 units per milligram have been reported for relatively pure specimens of penicillin K. When injected into the animal body in prophylactic or protective tests a reversal in comparative activity occurs, however, and penicillin K may be only one-half to one-tenth as active against the infecting organism as penicillin G (1). In a recent article Coghill, Osterberg and Hazel (2) state that preliminary experimental evidence indicates that "penicillin K is so unstable in the human system that its therapeutic usefulness when used parenterally is open to question." Penicillin K has been further implicated by numerous groups as being probably responsible for the adverse trend noted in the rapid treatment of syphilis with penicillin.

At the present time a large amount of investigational work is in progress designed toward determining the fate of penicillin K in the animal body as a possible answer to its apparent ineffectiveness when compared to penicillin G. Numerous theories have been advanced concerning the reasons and many of them are quite plausible although not entirely proved. One theory is that penicillin K is selectively inactivated by an enzyme system found in the circulating blood and active both *in vivo* and *in vitro*. A second theory is that biological conversion and excretion as an inactive compound may occur while still a third theory is that certain organs may be able to metabolize selectively penicillin K to a more marked extent than penicillin G. Actually any one or a combination of all might well be implicated but the final answer must await the results of further study.

A chance observation made by one of us in 1944 that preliminary injection of small doses of a mercurial diuretic resulted in a marked diminution in duration of blood levels following injections of penicillin while administration of doses sufficiently large to produce anuria resulted in prolongation of blood levels for as much as forty-eight hours suggested a possible line of attack to the problem concerned with *in vivo* destruction or inactivation of penicillin K. If one were to assume that the poorer clinical results were due to the destruction of penicillin K to a much greater extent than occurred with penicillin G in a given time period, the prevention of excretion should produce but little change in the over-all blood level picture as compared with that obtained in normal animals following parenteral administration of these two penicillins. With this in mind the following series of experiments were conducted to determine whether a rapid and complete inactivation of penicillin K occurred when the main organs of excretion were excluded from the circulation or

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whether a marked difference could be demonstrated in the prolongation of effective serum levels of penicillins G and K.

EXPERIMENTAL

In conducting these experiments healthy rabbits weighing approximately 3 Kg. were selected and placed under anesthesia by administering 200 mg./Kg. of sodium barbital supplemented by ether during the actual operative procedure. The kidneys were exposed as rapidly as possible and a common ligature of heavy silk was placed around the renal artery, the renal vein, and the ureter in such a manner that the kidney was completely isolated. The appropriate amount of freshly prepared solutions of the crystalline penicillin was then administered by way of the marginal ear vein and blood samples were removed by cardiac puncture at 5, 30, 60, 120, 180, 240, and 300 minutes for the determination of serum penicillin levels. Estimated dilutions of the serum were made in 1% phosphate buffer (*p*H 6.0) and the penicillin content determined by the *Staph. aureus* plate-filter disc method.

In carrying out these experiments a total of fourteen rabbits was employed, five each receiving penicillins G and K at a dose level of 20,000 units per kilogram and two each receiving the penicillins at a dose level of 2000 units per kilogram. The results appear in tabular form in Table I and are graphically illustrated in Figs. 1 and 2.

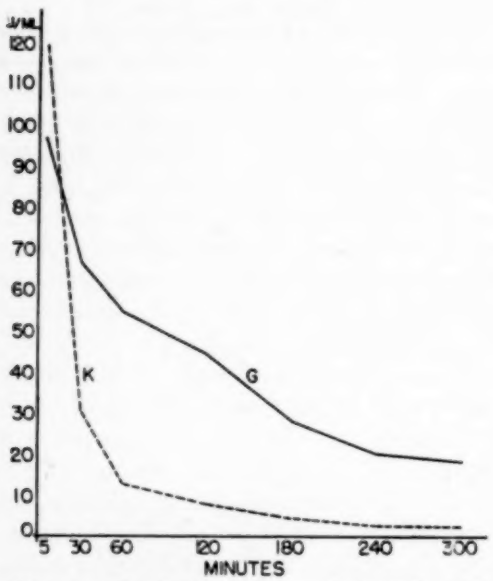

Fig. 1.—Blood levels in renal ligated rabits following I.V. injections of penicillin G and K using a dose of 20,000 U/Kg.

DISCUSSION

An inspection of the table and figures reveals that complete renal ligation in rabbits results in a marked prolongation of penicillin blood levels. Penicillin G produced blood levels which, in general, were much higher than those produced by penicillin K. The important role that the kidneys play in elimination of penicillins from the body is well illustrated since in the normal rabbit receiving these dosages, penicillin would not be detectable after two or three hours. It is evident, however, that the theory that penicillin K is rapidly and completely destroyed by sera or organs (other than the kidney) is not supported by this data. As long as four hours after the intravenous injection of 2000 units per kilogram in renal ligated rabbits, a dose comparable to 120,000 units in a 60-Kg. man, the blood level for penicillin K averages 0.63 unit/ml. of serum. Since the level for penicillin G is 2.22 units/ml. four hours after injection of a single dose, it is evident that definite inactivation of penicillin K is obtained although this is far from complete. As would be expected, the injection of 20,000 units per kilogram produced extremely high levels in these renal ligated animals. At this dosage level over 2 units of penicillin K per ml. of serum were found five hours after injection.

TABLE I.—AVERAGE BLOOD LEVELS OF CRYSTALLINE PENICILLIN G AND K IN RENAL LIGATED RABBITS

Time Interval, Min.	Penicillin G, 20,000	Penicillin K, 20,000	Penicillin G, 2000	Penicillin K, 2000
5	96.8[a]	120.0[a]	17.25[a]	8.85[a]
30	65.7	30.3	7.25	2.42
60	54.3	12.9	6.75	1.95
120	44.6	7.7	5.12	0.86
180	28.0	4.7	2.72	0.73
240	20.8	2.5	2.22	0.63
300	18.3	2.2	2.17	[b]

[a] Units per ml. of serum.
[b] Animals dead.

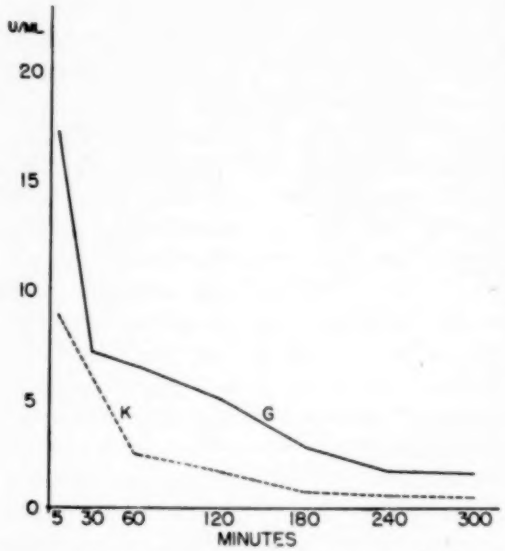

Fig. 2.—Blood levels following I.V. injection of 2000 U/Kg. of penicillin G and K in renal ligated rabbits.

It is apparent from these figures that the failure of penicillin K to maintain adequate blood levels is only partially due to destruction or inactivation by some mechanism in the body and that excretion by the kidney plays an important role as well. In a large series of excretion experiments which are being reported elsewhere the kidneys were left intact. In these cases no demonstrable penicillin serum levels were found after thirty minutes when 2000 units per kilogram of penicillin K were injected while one hour after injection of 20,000 units per kilogram only 1.5 units per ml. of serum were found. By way of comparison 12.0 u./ml. of serum were found one hour after injection of 20,000 u./Kg. of penicillin K in the renal ligated animals.

It should be noted (Table I) that from the second to the fourth hours the blood levels of penicillin K following injection of 2000 units per Kg. are practically constant. At the second hour (120 minutes) 0.86 unit per ml. of serum were found, at the third hour 0.73 unit per ml., and at the fourth hour 0.63 unit per ml. Similarly, following injection of 2000 units/Kg. of penicillin G serum levels of 2.72 units per ml., 2.22 units per ml., and 2.17 units per ml. were obtained at the third, fourth, and fifth hour, respectively. Both penicillin fractions are markedly depleted during the first two hours. If this depletion in activity is due to serum inactivation or some other inactivating medium it is difficult to explain why these penicillin fractions maintain a fairly constant level for from two to three hours unless it is explained on the basis that the effective mechanism is exhausted following contact with certain quantities of penicillin. It is apparent however, that serum inactivation of penicillin K is not the complete answer to its lower therapeutic efficiency since, even though one can demonstrate inactivation of certain quantities of penicillin K both *in vitro* and *in vivo*, in the ligated rabbit penicillin K in a concentration of less than one unit per ml. was maintained for a period of at least two hours.

In rabbits receiving either 20,000 units/Kg. or 2000 units/Kg. of penicillin K a rapid initial drop in the blood levels occurred when compared with those rabbits receiving similar doses of penicillin G, while at later periods the rate of decline was more gradual for both fractions with penicillin G falling off more rapidly between thirty minutes and five hours. This rapid initial fall in the serum level of penicillin K indicates that this type of penicillin is probably inactivated more rapidly than is penicillin G under identical circumstances. There is also the possibility that some other route of excretion may be involved. A few preliminary experiments indicated that in normal animals, following injections of both penicillins K and G, penicillin K levels in the bile were three times as high as those obtained with penicillin G. In view of the fact that the flow of bile in any thirty-minute period could not be a large volume it is hardly possible that this offers an adequate explanation but it may be that this is an indication that more penicillin K may be withdrawn from the system by the liver than is the case with penicillin G and that some type of inactivation occurs in the living animal within the liver itself.

SUMMARY

Complete renal ligation in rabbits results in marked prolongation of blood levels following the injection of penicillin K as well as penicillin G. The theory that a combination of excretion and inactivation may be involved in the failure of penicillin K to maintain adequate blood levels in rabbits is advanced. Available data indicate the presence of an undetermined inactivation mechanism which inactivates both penicillins to some extent but which inactivates K to a greater extent. The probability that this mechanism may be exhausted by large doses of either form of penicillin is suggested.

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The Determination of Arsenic in Official Arsenicals*,†

By KENNETH L. WATERS, RUSSELL J. KELLY, and GEORGE D. BEAL

An assay for the determination of arsenic is presented which is applicable to six of the seven Pharmacopoeial arsenicals. The method employs an alkali-acid permanganate oxidation for the destruction of organic matter.

THE USE OF potassium permanganate for the decomposition of organic compounds prior to determination of their inorganic constituents is well known. Generally, this oxidation is performed in a strongly acid medium; however, Leclercq (1) showed that an alkaline permanganate solution could be successfully employed to decompose certain types of iodo-organic compounds. Butler and Burdett (2) applied this procedure to the decomposition of tetraiodophthalein with such excellent results that the U. S. P. XIII (3) has recognized an alkaline permanganate oxidation as official in the assay of a number of compounds containing iodine. Clark and Jones (4) demonstrated that many of the acid- or alkali-soluble iodine-containing organic compounds could be decomposed if given an alkaline permanganate treatment followed by an acid permanganate treatment. Recently, Jenkins, Waters, and Beal (5) reported an alkaline permanganate oxidation for the determination of chlorine in 2,2′-dihydroxy-5,5′-dichlorodiphenylmethane. Since the official arsenicals are alkali-soluble it would seem that the decomposition of these products should be no more difficult than that of the iodine compounds. If such a decomposition procedure could be rendered applicable to the official products it would permit further unification of U. S. P. assays thus eliminating the four different methods now in use.

We have found that six of the seven official arsenicals are indeed easily decomposed by rather mild oxidation. We first employ an alkaline solution of potassium permanganate and then complete the oxidation in acid solution. The results obtained agree closely with those obtained by presently official Pharmacopœial assay procedures.

* Received Oct. 28, 1946, from the Department of Research in Pure Chemistry, Mellon Institute of Industrial Research, Pittsburgh, Pa.

† This research was done with the assistance of a grant from the Board of Trustees of the United States Pharmacopœial Convention.

EXPERIMENTAL

The method which we have found to give very satisfactory results on the determination of arsenic in official arsenicals is as follows:

Place about 150 mg. of the sample, accurately weighed, in a 500-cc. glass-stoppered flask, and add 15 cc. of sodium hydroxide T. S. Add 25 cc. of potassium permanganate (1 in 15) and several glass beads, then boil gently for ten minutes. Cautiously add 25 cc. of sulfuric acid (1 in 2) and again boil gently for ten minutes. Add hydrogen peroxide T. S. dropwise to reduce the brown precipitate and the excess of potassium permanganate. Wash down the sides of the flask and boil gently for ten minutes or until the excess hydrogen peroxide is destroyed. Cool, and add $N/10$ potassium permanganate dropwise to produce a slight pink color, then just discharge the pink color by adding dropwise $N/10$ oxalic acid. Add about 50 cc. of water and 20 cc. of potassium iodide T. S., stopper the flask, and allow to stand in a cool, dark place for one hour. Titrate the liberated iodine with $N/10$ sodium thiosulfate without the use of starch indicator. Each cubic centimeter of tenth-normal sodium thiosulfate is equivalent to 3.746 mg. of As.

In Table I are recorded the results obtained upon analysis of six of the seven Pharmacopœial arsenicals.

TABLE I.—ARSENIC IN OFFICIAL ARSENICALS

	Per Cent Arsenic	
	U. S. P. XIII Assay	New Method
Arsphenamine	33.24(2)[a]	33.29(4)
Neoarsphenamine	20.79(2)	20.66(4)
Dichlorophenarsine	26.52(2)	26.55(5)
Carbarsone	28.42(2)	28.28(4)
Sulfarsphenamine	21.91(2)	22.03(5)
Tryparsamide	25.47(2)	25.44(4)

[a] Numbers in parentheses indicate the number of determinations made.

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Comparison of Several Methods for the Determination of Arsenic in Pentavalent Arsenicals Alone and in Combination with Bismuth*

By PAUL S. JORGENSEN

A number of methods for determining arsenic have been studied to ascertain their applicability to the determination of arsenic in pentavalent arsenicals, and in preparations containing both pentavalent arsenicals and bismuth compounds. Of the methods studied, it was found that the official method of the Association of Official Agricultural Chemists gave more accurate and consistent results under all conditions than any other procedure.

THE U. S. P. XII recognizes three pentavalent arsenicals, Carbarsone, Sodium Cacodylate, and Tryparsamide, and New and Nonofficial Remedies, 1946 Edition, describes two of these together with Acetarsone and Phenarsone Sulfoxylate. Others are sometimes encountered under private trade names. Most of these compounds are closely related chemically to arsanilic acid. Others are alkyl pentavalent arsenicals. A reliable method for the determination of arsenic is essential in the establishment of the strength and quality of these preparations and it seems desirable to have one method which will apply to all of them.

Rupp and Lehmann (1) described a method for the determination of arsenic based on the oxidation of organic matter with potassium permanganate and sulfuric acid, and the subsequent addition of potassium iodide and titration of the liberated iodine. This method has been adopted by the U. S. P. XII for the assay of Carbarsone and the trivalent arsenicals, Arsphenamine, Neoarsphenamine, and Sulfarsphenamine. The New and Nonofficial Remedies recommends this method for the analysis of Silver Arsphenamine and Acetarsone. A method in which the oxidation of organic matter is accomplished with hydrogen peroxide and sulfuric acid and the arsenic determined, after reduction with hydrazine sulfate, by oxidation with potassium bromate, has been adopted by the U. S. P. XII Supplement for the estimation of arsenic in Dichlorophenarsine Hydrochloride and Oxophenarsine Hydrochloride and by N. N. R. for the assay of Phenarsone Sulfoxylate. The Association of Official Agricultural Chemists has described a method (2) in which the arsenic is determined by direct oxidation with iodine after digestion with potassium sulfate, starch, and sulfuric acid. This procedure has been adopted by the U. S. P. XII for Sodium Cacodylate and by the N. F. VII for Ampuls of Sodium Cacodylate. Little, Cahen, and Morgan (3) used fuming nitric acid for destruction of organic material, reduced the arsenic with potassium iodide and reoxidized it for the quantitative estimation with standard iodine solution. This method is described in the U. S. P. XII for the determination of arsenic in Tryparsamide. Newbery (4) used a similar procedure except that he employed ammonium persulfate for destruction of the organic material. Kahane (5) used a mixture of sulfuric, nitric and perchloric acids as oxidizing agents, but this method was not studied due to the danger attached to the use of perchloric acid.

In order to test the reliability of the various methods described, a series of arsenic determinations were made on a representative number of pure organic arsenicals, and on a mixture of known amounts of sodium cacodylate, sodium arsanilate, and arsanilic acid with approximately 100 mg. of bismuth subgallate since these arsenicals are frequently combined with bismuth salts including bismuth subgallate in commercial preparations. Some of the methods gave accurate and consistent results when applied to the pure compounds, but with bismuth added most methods are generally not applicable, due to the development of colored solutions, indefinite end points in the titration, or to variable blanks on reagents. The

* Received Nov. 26, 1946, from the Chemical Section of the Medical Division, Food and Drug Administration, Federal Security Agency, Washington, D. C.

results of these determinations are recorded in Table I and Table II. Table III gives results on some commercial preparations of arsenicals combined with bismuth.

and violet colors developed during the titration before the end point was reached. The end point was represented by a change from the brown or violet color to a distinct blue

TABLE I.—RESULTS OF ANALYSIS OF PURE ORGANIC ARSENICALS BY FIVE RECOGNIZED METHODS

Sample	A. O. A. C. Method, % As	U. S. P. XII Tryparsamide Method, % As	U. S. P. XII Arsphenamine Method, % As	U. S. P. Suppl. Dichlorophenarsine Method, % As	Newbery's Method, % As
Sodium arsanilate	25.63	25.95	22.46	26.2	20.95
As, 25.6%	25.7	25.9	24.14	26.11	20.84
Carbarsone	28.81	28.35	28.96	28.73	29.64 29.0
As, 28.81%	28.87	28.68	29.44	28.41	29.88 29.07
Tryparsamide	25.45	25.65	25.46	26.03	25.15
As, 25.31%	25.49	25.31	27.28	25.99	25.7
Acetarsone	27.28	27.81	28.5	28.0	27.79 27.65
As, 27.24%	27.28	27.64	28.41	28.79	27.91 25.28
Phenarsone sulfoxylate	17.42	17.76	18.5	17.95	17.6
As, 17–18.5%	17.37	17.74	18.4	17.91	17.91
Stovarsol Sodium	22.7	22.91	23.7	23.21 19.36	22.66 22.79
	22.56	22.7	23.76	22.97	21.9 22.77
Anhyd. sodium cacodylate	46.63	47.1	....	48.16	45.44
As, 46.82%	46.73	47.43	....	49.2	43.7
Arsanilic Acid	34.75	34.39	35.17	35.98	34.89
As, 34.52%	34.47	35.29	34.83	36.03	34.85
Chloroarsenol[a]	93.05	96.87	101.04	98.61	102.1
	93.05	96.11	102.5	97.91	100
Acetylarsan[a]	99.0	99.0	104.25	101.25	100.5
	99.0	99.75	104.25	102.0	101.25

[a] Per cent of declared amount in ampul solution.

TABLE II.—RESULTS OF ANALYSIS OF PURE ORGANIC ARSENICALS WITH BISMUTH SUBGALLATE

Sample	A. O. A. C. Method, % As	U. S. P. XII Tryparsamide Method, % As	U. S. P. XII Arsphenamine Method, % As	U. S. P. Suppl. Dichlorophenarsine Method, % As	Newbery's Method, % As
Sodium cacodylate	46.52	44.11	.....	48.3	.....
As, 46.82%, with bismuth	46.68	42.27	Colored soln.	48.8	Colored soln.
Arsanilic acid	34.24	33.71	.....	34.62	.....
As, 34.52%, with bismuth	34.52	28.29	Colored soln.	34.83	Colored soln.
Sodium arsanilate	25.12	25.49	.....	25.92	.....
As, 25.6%, with bismuth	25.18	25.7	Colored soln.	25.73	Colored soln.

TABLE III.—ARSENIC FOUND IN PER CENT OF DECLARED AMOUNT IN COMMERCIAL AMPUL SOLUTION CONTAINING BISMUTH

Sample	A. O. A. C. Method	U. S. P. XII Tyrparsamide Method	U. S. P. XII Arsphenamine Method	U. S. P. Suppl. Dichlorophenarsine Method	Newbery's Method
1	96.44	93.12	.....	105.62	.....
	96.44	91.56	Colored soln.	106.25	Colored soln.
2	101.2	102.19	.....	105.94	.....
	100.6	104.06	Colored soln.	105.94	Colored soln.
3	91.07	88.93	.....	95.36	.....
	91.07	87.03	Colored soln.	83.84	Colored soln.
4	116.25 116.87	121.2 98.5	.....	123.5	.....
	116.87	112.5 82.5	Colored soln.	111.25	Colored soln.
5	100.18	102.86	.....	105.53	.....
	100.18	103.93	Colored soln.	104.47	Colored soln.

DISCUSSION

Results by the A. O. A. C. Method gave values for arsenic which were in close agreement with the theoretical amount. Brown

when one drop of standard iodine solution was added. This change was quite sharp and easily detected.

The results by the U. S. P. Method for

arsenic in tryparsamide were reasonably good in the absence of bismuth. The method calls for boiling with potassium iodide to reduce arsenate to arsenite. Iodine was liberated by this treatment and the last traces not removable by boiling were removed by the addition of a few drops of dilute thiosulfate solution. In the presence of bismuth it was not unusual to have solutions in which the color was not due to iodine and since it was impossible to tell what portion of the color, if any, was attributable to iodine, an accurate titration could not be made. This no doubt was the cause of the variable results obtained.

Glycart (6) in his report on methods for determining arsenic in sodium cacodylate pointed out that the U. S. P. Method for Arsphenamine was not suitable for sodium cacodylate. This statement has been confirmed and the results indicate that the method may tend to give high results on other arsenicals. It was found to be inapplicable to preparations containing bismuth due to the development of colored solutions which made titration impossible.

Results by the U. S. P. Method for Dichlorophenarsine were found to be quite variable. Blank determinations on the reagents were variable and abnormally high which may account for these discrepancies. The blank determinations were not used in making calculations reported in the table.

Values for arsenic obtained by Newbery's Method on some of the pure arsenicals are not in accord with the theoretical values. When bismuth is present with arsenic, measurement of the arsenic with standard iodine solution is impossible due to the formation of colored solutions.

CONCLUSIONS

Several methods for the determination of arsenic in organic pentavalent arsenicals have been compared. The results obtained by the present official A. O. A. C. Method for arsenic in sodium cacodylate on pure organic compounds and on sodium cacodylate, sodium arsanilate, and arsanilic acid in combination with bismuth justifies the conclusion that this method is more generally applicable to the determination of arsenic in pentavalent arsenicals than the other methods studied.

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WHO MAKES IT?

The National Registry of Rare Chemicals, Armour Research Foundation, 33rd, Federal and Dearborn Streets, Chicago, Ill., seeks information on sources of supply for the following chemicals:

Talomucic acid
Thebaine
Alkannin
2-Pyridine aldehyde
N-Piperidine acetaldehyde
l-Phenylalanine
2,3-Dimethylbenzaldehyde
3-Pyridine aldehyde
1,18-Octadecandioic acid
Nucleoproteins
Arterenol
o,p-Dihydroxydiphenyl
Cinchophen amyl ester
2-Phenylbenzopyrone
3-Chlorotetrahydrofuran
d-Camphor
l-Menthol
Trypsin, crystalline
Thujone
Pulegone
4-Amino-2,2′-dichlorobenzophenone
4-Amino-2,4′-dichlorobenzophenone
2-Hexene-1-ol
Kynurenic acid

A Study of the Toxicity of Two Isomeric Sodium Hydroxymercuribenzoates*,†

By THOMAS J. HALEY‡,§ and L. D. EDWARDS‖,¶

The LD_{50} (intraperitoneal) for sodium *p*-hydroxymercuribenzoate for rats was determined to be 5 mg./Kg. The LD_{50} (intraperitoneal) for sodium *o*-hydroxymercuribenzoate for rats was determined to be 15.5 mg./Kg. Chronic toxicity determinations following intraperitoneal injections in rabbits showed that both compounds were nontoxic under the conditions employed. Hematological examinations as a means of following chronic mercurial toxicity in rabbits yielded no results with the concentrations studied. An oral toxicity determination, employing the *para* compound in the concentration suggested for a mouthwash, showed that it was nontoxic to rabbits even when given in large amounts.

Hanke (1), in 1940, reported that sodium *p*-hydroxymercuribenzoate was a good oral germicide. However, no mention was made of the possibility of mercury toxicity from the use of this chemical. Further, the literature shows no data on the toxicity of the *ortho* isomer of this compound.

Acute mercury toxicity shows tissue damage which differs considerably from that produced by the chronic type of mercurialism [Sollmann (2)]. Studies of chronic mercury toxicity in rabbits have been reported by several investigators (3–8). These studies followed the course of mercurial toxicity by changes in the chemical constituents of the blood. Other investigators have followed mercurial toxicity by hematological changes. Miyake (9) found that the red and white cells increased when mercuric chloride was given as a daily injection of less than the M. L. D. Lo Cascio (10) reported that intravenous injections of several mercurials caused no rapid erythroblastic reaction, but that toxic doses caused a decreased number of red cells with the appearance of nucleated cells. Further, leucocytosis with an inversion of the cell ratio occurred in twenty-four hours.

The purpose of this study was to determine the toxicity of both isomeric sodium hydroxymercuribenzoates.

EXPERIMENTAL

Acute toxicity following intraperitoneal injection was studied in rats. Exploratory dosage with the *para* isomer, employing twenty-two animals, showed that the M. L. D. range was between 5.0 and 6.0 mg. per Kg. body weight. A similar determination with the *ortho* isomer, using nineteen animals, showed that its M. L. D. range was between 14.0 and 16.0 mg. per Kg. body weight. Following this exploratory work the LD_{50} was determined by the method of Wright (11). The results for both isomers are shown in Table I.

TABLE I.—THE DETERMINATION OF THE LD_{50} OF HYDROXYMERCURIBENZOATES IN RATS (INTRAPERITONEAL)

Isomer	Dose, Mg./Kg.	Experimental Alive	Experimental Dead	Integrated Alive	Integrated Dead	Mortality, %
Para	4.5	4	6	17	6	26.0
	5.0	2	8	13	14	51.8
	5.5	2	8	11	22	66.6
	6.0	3	7	9	29	76.3
	6.5	3	7	6	36	85.7
	7.0	3	7	3	43	93.4
	Time of reading—120 hours. LD_{50} 4.96 mg./Kg.					
Para	5.0	3	6	9	6	40.0
	5.5	2	7	6	13	68.4
	6.0	2	7	4	20	83.3
	6.5	2	7	2	27	93.3
	Time of reading—120 hours. LD_{50} 5.17 mg./Kg.					
Ortho	14.5	9	0	23	0	00.0
	15.0	8	1	14	1	6.6
	15.5	4	5	6	6	50.0
	16.0	2	7	2	13	86.6
	Time of reading—192 hours. LD_{50} 15.5 mg./Kg.					

* Received Aug. 5, 1946, from Purdue University School of Pharmacy, Lafayette, Ind.
Presented to the Scientific Section, A. PH. A., Pittsburgh meeting, 1946.,
†A chemical study on this subject was published in THIS JOURNAL, 35, 179–81(1946).
‡ Abstracted from a dissertation submitted to the Graduate Council of the University of Florida by Thomas J. Haley in partial fulfillment of the requirements for the degree of Doctor of Philosophy. Additional material not in the dissertation is also included.
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‖ Professor of pharmacology, Purdue University School of Pharmacy.
¶ The authors are indebted to Dr. P. A. Foote for the first sample of the *para* compound and for the mouthwash containing it as the active ingredient. The second sample of the *para* compound and the *ortho* compound were prepared and analyzed by the authors.

In the first determination with the *para* isomer and that of the *ortho* isomer the rats were all males; in the second determination with the *para* isomer the sexes were evenly distributed and the survivors were the same. This would indicate that sex plays little or no part in the acute toxicity caused by the *para* compound.

Features which are common to all determinations are the appearance of a transitory hind quarters paralysis immediately after injection and the development of an anorexia after twenty-four hours. The anorexia was not permanent as the survivors appeared to eat and drink normally after the completion of the test.

Chronic toxicity following intraperitoneal injection in rabbits was determined by following the hematological changes in the blood for a period of six weeks. Injections of 10 cc. per Kg. body weight of a 1:5000 solution of the isomers (concentration suggested for the mouthwash) were given weekly after which a complete hematological examination was made. The controls received 10 cc. of normal saline solution per kilogram. The results of this series of experiments are shown in Table II.

The figures for the hematological constitutents of rabbit's blood as given in the table as well as the eosinophil and basophil counts were entirely within the normals as recorded by Kolmer and Boerner (12).

TABLE II.—THE DETERMINATION OF THE CHRONIC TOXICITY OF HYDROXYMERCURIBENZOATES IN RABBITS

Animal	Starting Weight, Kg.	Final Weight, Kg.	Weight Gain, Kg.	Starting Erythrocytes	Final Erythrocytes	Average Erythrocytes
241 Control	0.582	1.390	0.807	4,850,000	7,170,000	6,058,000
244 Control	0.619	1.318	0.699	5,810,000	7,090,000	4,988,000
207 Control	0.626	1.152	0.526	5,830,000	5,970,000	4,988,000
215 Control	0.820	1.615	0.795	5,320,000	5,020,000	4,976,000
216 *Para*	0.626	1.649	1.023	6,170,000	7,080,000	5,944,000
232 *Para*	0.657	1.400	0.743	3,670,000	7,060,000	5,782,000
210 *Para*	0.763	1.376	0.613	5,300,000	7,040,000	6,052,000
208 *Para*	0.487	1.325	0.838	7,050,000	7,040,000	6,446,000
227 *Ortho*	0.668	1.222	0.554	5,600,000	4,540,000	5,138,000
234 *Ortho*	0.619	1.550	0.931	5,110,000	5,140,000	5,664,000
206 *Ortho*	0.726	1.448	0.722	4,320,000	6,260,000	5,724,000
225 *Ortho*	0.663	1.441	0.778	5,970,000	6,030,000	5,408,000

Animal	Starting Leucocytes	Final Leucocytes	Average Leucocytes	Average Neutrophils	Average Lymphocytes	Average Monocytes
241 Control	4000	6900	6380	4500	1800	300
244 Control	6900	7400	6070	5000	1000	325
207 Control	6300	7050	6200	5100	1800	350
215 Control	4650	5150	6230	4500	1700	290
216 *Para*	6300	6800	6760	5000	1000	350
232 *Para*	9150	4800	6500	6000	1900	500
210 *Para*	9050	7150	6140	5800	1850	460
208 *Para*	7000	4550	6990	3500	4500	350
227 *Ortho*	7950	5350	6350	6000	1500	300
234 *Ortho*	5400	9600	6470	5500	2500	400
206 *Ortho*	5350	6100	6370	5000	1000	325
225 *Ortho*	5700	9550	6920	6000	1500	350

TABLE III.—EFFECTS OF ORAL ADMINISTRATION OF *p*-HYDROXYMERCURIBENZOATE IN RABBITS

Animal	Starting Weight, Kg.	Final Weight, Kg.	Weight Gain, Kg.	Dose/ Kg., Cc.	Total Time, Days	Amount Given, Cc.	Mercury Received, Mg.	Results
1	1.238	1.238	0.000	1.5	1	1.85	0.21	Died of pulmonary edema
2	1.528	2.231	0.703	1.5	62	180.9	20.12	Intestines, spleen, kidneys, and liver normal. Animal appeared normal
3	1.061	1.834	0.773	1.5	62	132.6	14.75	Intestines, spleen, kidneys, and liver normal. One lesion in stomach otherwise normal
4 Control	0.887	1.550	0.663	...	62	....	...	Intestines, spleen, kidneys, and liver normal. Animal appeared normal
5 Control	1.384	1.900	0.516	...	62	....	...	Intestines, spleen, kidneys, and liver normal. Animal appeared normal
6	1.485	1.207	−0.278	3.0	10	38.6	4.29	Died of pneumonia and peritonitis. Lungs dissolved and intestines inflamed
7	1.559	2.180	0.621	3.0	30	164.4	18.28	Intestines, spleen, kidneys, and liver normal. Animal appeared normal
8	1.536	2.074	0.538	3.0	30	168.6	18.75	Intestines, spleen, kidneys, and liver normal. Animal appeared normal

This showed that the compounds had very little or no effect upon the hematological constituents of the rabbit's blood. Further, the compounds caused no stimulation of the blood-forming tissues in the body.

As the above dosage was not fatal the animals were sacrificed and the organs examined macroscopically. No tissues of the experimental animals showed any differences from those of the controls. It is possible that the isomers are rapidly detoxified and excreted from the body so that the usual symptoms of chronic toxicity would not be seen except over a much longer period of administration of the compounds.

Inasmuch as the original use of the *para* compound was as an oral germicide, it was decided that a determination of the effects of oral ingestion of the solution should be carried out. One group of rabbits received daily doses of 1.5 cc. of a 1:5000 solution per kilogram body weight by stomach tube. At the end of thirty days no evidence of toxicity was apparent. A second group of rabbits received 3.0 cc. of the same solution per kilogram body weight in the same manner. At the end of the second thirty-day period all animals were sacrificed and the organs examined macroscopically. The results are given in Table III.

From the results (Table III) it may be seen that none of the usual symptoms of chronic mercury poisoning were present, and further that the organs showed no lesions or other pathological degenerations which could be attributed to the mercury in the compound.

CONCLUSIONS

1. The LD_{50} (intraperitoneal) for sodium *p*-hydroxymercuribenzoate for rats was shown to be 4.96 mg. per Kg. The LD_{50} (intraperitoneal) for sodium *o*-hydroxymercuribenzoate for rats was shown to be 15.5 mg. per Kg.

2. Chronic toxicity determinations following intraperitoneal injections in rabbits showed that both compounds were nontoxic under the conditions employed.

3. Hematological examinations as a means of following chronic mercurial toxicity in rabbits yielded no results with the concentrations studied.

4. An oral toxicity determination, employing the *para* compound in the concentration suggested for a mouthwash, showed that the compound was nontoxic to rabbits even when given in large amounts.

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